PIXEL PATHOLOGY:
digital workflow and computational analysis

Alexi Baidoshvili

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digital workflow and computational analysis

Pixel pathologie:
digitale workflow en computationele analyse

(met samenvatting in het Nederlands)

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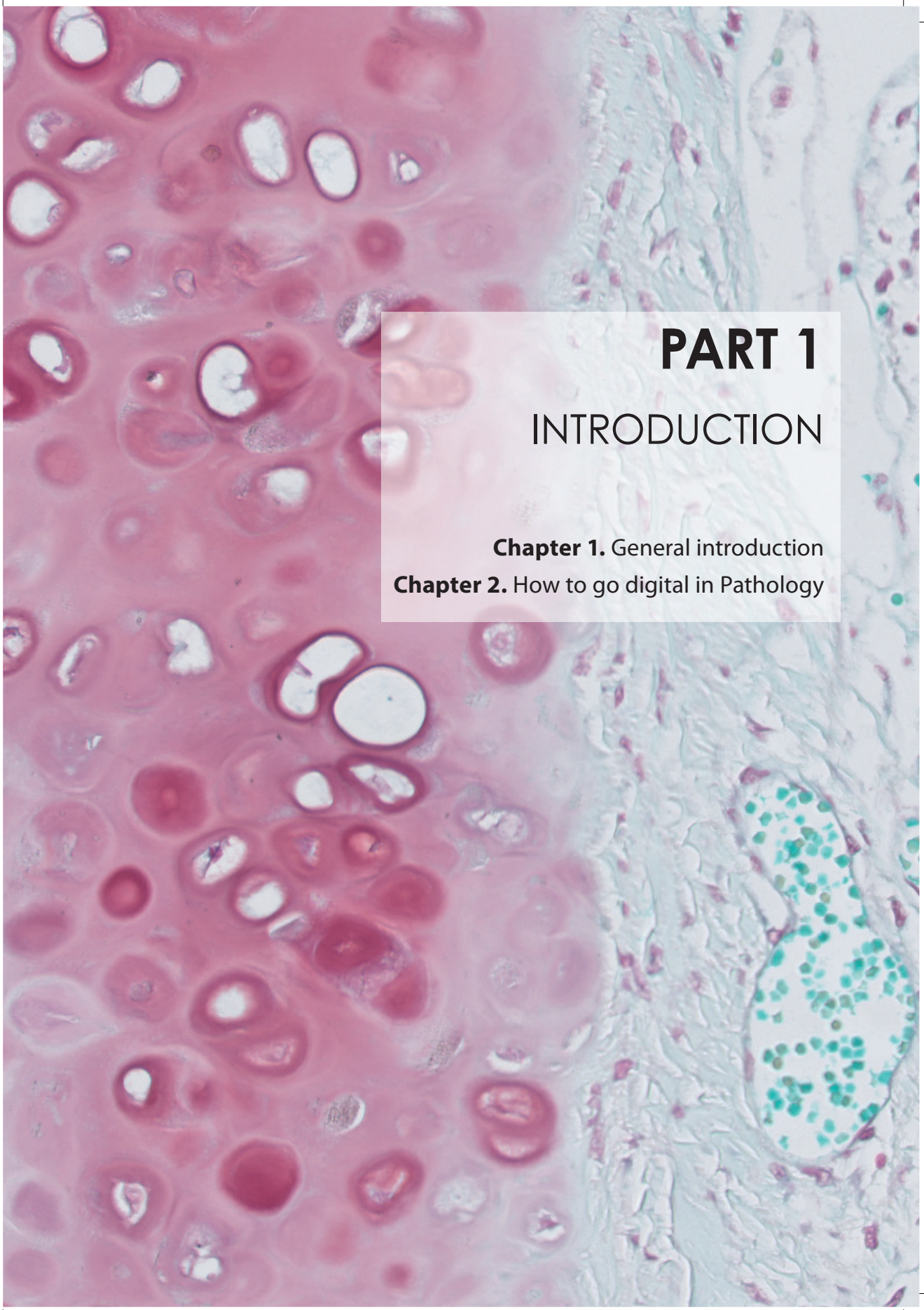
CONTENTS



PART 1

INTRODUCTION

CHAPTER 1

General introduction

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GENERAL INTRODUCTION

The microscope was invented and developed by the Dutchmen Sacharias Janssen and Antoni van Leeuwenhoek around the 17th century. They made their discoveries with a microscope that initially had two pieces of ground glass and later only one piece of cut glass. A single lens, after all, was much less affected by distortion and chromatic aberration than a compound microscope. This invention initiated centuries of light microscopic studies of tissues in health and disease and is still very relevant for today's health care. Microscopy has been an indispensable tool for the pathologist to study the architecture of tissues and cells during diagnostics of diseases, and has been developed from light microscopy to fluorescence and confocal laser scanning microscopy.

The introduction of video technology and digital cameras allowed to create digital microscopic images since the 80ies. Already in 1986 "telepathology" was made possible after the introduction of video cameras mounted on microscopes, making it possible for live images to be shared with people at different locations. This allowed live teleconsultation and remote diagnosis of frozen sections, although at relatively low resolution. A little later, affordable digital cameras became available, allowing efficient capturing of still digital images at high resolution. This unlocked new possibilities for both pathologists and researchers alike, with the use of image analysis and smart software that provided new insights and helped to work more efficiently, and increase accuracy and reproducibility. However, in view of camera and computer speed restraints, analysis was usually limited to a few snapshots, making image analysis selective and impractical in daily practice.

In the last decade, digital slide scanners allowing to completely digitize pathology sections (whole slide images, WSI) at high resolution and acceptable speed were introduced which slowly made their way into pathology labs as a "digital age" alternative to the conventional microscope (1, 2, 3, 4).

"Digital pathology" has been successfully implemented around the world for education, clinicopathologic conferences and research. Its adoption for primary diagnostics is increasing but has been primarily limited to specific, generally low-volume niches, such as second opinions and frozen sections. Digital pathology is gaining more and more interest worldwide and several pilot initiatives show that the time has come to fully integrate digital diagnostics. For instance, in Scandinavia (5, 6), and in Canada – through the Canadian Association of Pathologists - there are now established guidelines for telepathology service for anatomic pathology (7). Some Dutch pathology laboratories in The Netherlands are leaders in the transition to work digitally.

At LabPON (Laboratory for Pathology East Netherlands), we started using WSI in 2010 and discovered right away that we needed to have a fully digital diagnostics workflow to take advantage of all the benefits. As one of the first laboratories worldwide that succeeded in digitizing the entire workflow, we realize that there are still challenges ahead of us. This huge innovation is not only beneficial for our institute, but for the whole field. To make the most of our experience, we want to share our insights with every department of pathology worldwide. This experience can help other laboratories to complete this process smoothly and as cost-effectively

as possible (1).

During implementation of a fully digital diagnostic workflow, we faced many challenges. When we started with digitalisation only a few laboratories had some experience with digital diagnostics, but none were fully digital and little information was at hand regarding implementation of digital diagnostics. The most important challenges are listed below:

1. Making a business plan and implementation plan
2. Adjustments in information technology (IT) infrastructure
3. Selecting a suitable scanner
4. Adjustments and changes in laboratory logistics
5. Adjustments in the workplace of pathologists
6. Design and safe use of external consultation and remote work
7. Integration of IMS (Image Management System) into LMS (Laboratory Information Management System).
8. Develop, validate and use image analysis software

   Several of these challenges are dealt with in the various chapters of this thesis.

1. Making a business plan and implementation plan

If a laboratory decides to go digital, it first needs to make a flow analysis. This is the most important step for digitalisation. The flow analysis determines how many scanners are needed, what needs to change in the network and the important things that have to be tackled for digitalization. The flow analysis can also help with the business plan. When we started with digitalisation we did not find any information about flow analysis and business cases and we had to start with digital diagnostics without business case. In our laboratory we used a flow analysis to investigate how digital diagnostics could ensure a faster workflow and turnaround time (see Chapter 4). We also compared diagnostic time of experienced digital pathologists using both methods microscope and digitally (see Chapter 5). This will allow other labs to make a business plan based on experience and not on assumptions.

2. Adjustments in IT infrastructure

Analysis and necessary adjustment of IT infrastructure is the first step after making the business and implementation plan. IT components are very important for digitalisation since the workflow needs to be fast and up all the time.

Further, the image server is very important. The necessary storage volume of the image server needs to be calculated. Since the server receives a lot of data from different scanners at the same time and many pathologists simultaneously stream images from the server, a very good server is needed to handle this heavy load.

In addition to a good server, storage is very important. It is important to know how much storage you need, depending on image volume, file size, and duration of storage.

Speed and bandwidth of the network is important. Good cables and switches are essential for digitization.

The desk of the pathologists need to be equipped with fast computers that allow fast streaming of the WSI, for which quality of the graphics board but especially processor speed turned out to be important.

These various elements are discussed in Chapter 2.

3. Selecting a suitable scanner

Scanners may have several options, but the specific local requirements are important: scanning volume and required speed will determine the number of scanners needed, and options like the required level of automation, the need to scan fluorescence, big slides and Z-stacks determine the type of scanner. Scanners should be easy to operate and integrate, provide good image quality, and price is of course an issue. We did extensive market research, tested several scanners and eventually validated a scanner in our laboratory (see Chapter 2).

4. Adjustments and changes in laboratory logistics

The benefits of digital pathology for workflow improvement and thereby cost savings in pathology, at least partly outweighing investment costs, are increasingly recognized. Only a few previous studies have addressed whether a digital workflow is or could be more efficient (8, 9). A diagnostic digital pathology infrastructure may facilitate substantial workflow improvements which may lead to higher efficiency, better utilization of resources, higher throughput and lower turnaround time of cases, and also a streamlined collaboration both within a single pathology laboratory and across organizational boundaries, and thereby lower costs (10, 11). To further strengthen the literature and contribute to evidence required to build a vital business case for digital pathology, we report on the time savings following the adoption of digital pathology at the LabPON in Chapter 4.

5. Adjustments in the workplace of pathologists

The transition from analogue to digital diagnosis potentially results in fewer ergonomic problems than working with the microscope (12, 13, 14), but digital pathologists must move images all day with a mouse, perhaps leading to RSI (repetitive strain injury). In collaboration with our partners, we developed a set of tools for different devices (see Chapter 2).

Monitors are important components of digitization. In LabPON we have experimented with different monitors and describe the results in Chapter 2.

6. Design and safe use of external consultation and remote work

Digitization creates new possibilities for performing diagnostics from a distance, making it even possible to do diagnostics from outside the organization, which may be a big benefit (15, 16). There are currently no official regulations for remote diagnostics, but in LabPON we implemented secure digital connections as described in Chapter 2.

Remote digital revision and consultation is an important use case of digital pathology and is

increasingly used in the USA and a few other countries but has not been widely studied (17, 18, 19). In cases where a consultation from an external expert pathologist is required, the pathology material (glass slides and possibly tissue blocks) needs to be sent back and forth, traditionally through mail. This is a complicated logistic process that easily takes up to two weeks or more, depending on mail logistics and the speed of the consulted expert. Moreover, it is labor intensive, expensive and is error prone as slides may be misplaced or broken during transportation, whereas patient reports with patient data can easily be taken from broken or opened packages during transportation, breaching patient privacy. During this interval, doctors and patients are eagerly awaiting the final report with the diagnosis, and in consequence treatment may considerably be delayed, increasing patient anxiety levels, and sometimes also deteriorating the condition of the patient. A similar process takes place when a patient is referred to another hospital, in which case it is customary to ship the pathology material to reassess (revise) the case for the following reasons: revision may correct errors, serves to translate the report into local usual and up-to-date language, and ensures that material is available for demonstration at multidisciplinary meetings and for comparison with new material, e.g. biopsies from metastases.

The biggest challenge of external consultations and referral is security in transmission of patient information. With the use of digital pathology and its ability to stream images (WSI) digitally for external consultations, it has become topical to send patient information securely to other laboratories. For this several options have been identified. LabPON has set up a regional network in collaboration with the University Medical Center Groningen (UMCG) and Hospital Isala in Zwolle as described in Chapter 3.

7. Integration of IMS into LMS

Digital pathology solutions speed up and simplify the access to the actual cases. Comprehensive bidirectional integration with a Laboratory Information Management System (LIMS) is essential to optimally benefit from the improvement in efficiency and quality that digital pathology solutions offer. The full and bidirectional integration of both systems warrants accurate matching of cases. Any change in one system will lead to appropriate matching in the other.

Bidirectional desktop integration between IMS and LIMS contributes to patient safety in several ways. Every manual interaction can potentially cause mix-ups and errors. For instance, manually entering a case number in a system creates a risk of making a report based on the wrong slides. This risk is reduced by automated linking based on scanned and barcode information. This prevents mix-ups in patient reports, which may have severe consequences in term of patients undergoing unnecessary surgery, and thereby leading to claims from the patients. Integration of IMS and LIMS systems may prevent such calamities, thereby also lowering insurance costs of the laboratory.

LabPON has implemented full bidirectional integration of LIMS and IMS as described in Chapter 2

8. Develop, validate and use image analysis software

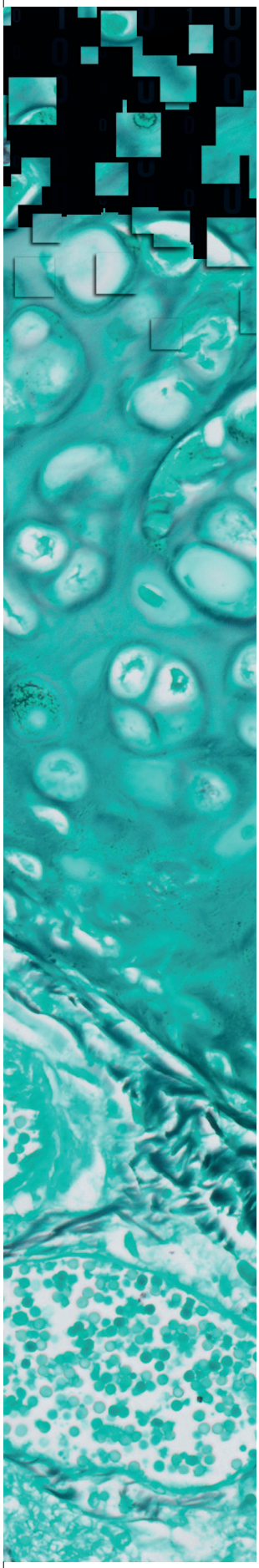
Introduction of digital pathology has led to revival of image analysis in pathology leading to renewed high expectations. Image analysis software can help a pathologist deliver efficient, fast and higher quality work. We expect these significant improvements in diagnosis:

- diagnostics will become more efficient
- diagnostics will become easier and higher in quality
- diagnosis will become more objective
- diagnoses will become more quantitative, opening new opportunities for improved quality control and research

LabPON collaborates with several partners to create better diagnostic applications. Recently, we investigated the potential of a deep learning approach for computer-aided quantification of intratumoural stroma in rectal cancer WSI as a proof of principle (see Chapter 6).

REFERENCES

1. Baidoshvili A. White paper; https://thepathologist.com/fileadmin/issues/App_Notes/0016-022-app-note-How_to_Go_Digital_in_Pathology.pdf
2. Pantanowitz L, Valenstein PN, Evans AJ, Kaplan KJ, Pfeifer JD, Wilbur DC, et al. Review of the current state of whole slide imaging in pathology. J Pathol Inform. 2011; 2:36. [PMCID: PMC3162745] [PubMed: 21886892]
3. Al-Janabi S, Huisman A, Van Diest PJ. Digital pathology: Current status and future perspectives. Histopathology. 2012; 61:1–9. [PubMed: 21477260]
4. Baak JP, van Diest PJ, Meijer GA. Experience with a dynamic inexpensive video-conferencing system for frozen section telepathology. Anal Cell Pathol. 2000; 21:169–75. [PubMed: 11339564]
5. Lundström C, Thorstenson S, Waltersson M, Persson A, Treanor D. Summary of 2nd Nordic symposium on digital pathology. J Pathol Inform 2015; 6:5.
6. Thorstenson S1, Molin J2, Lundström C3. Implementation of large-scale routine diagnostics using whole slide imaging in Sweden: Digital pathology experiences 2006-2013. J Pathol Inform. 2014 Mar 28; 5:14. doi: 10.4103/2153-3539.129452. eCollection 2014.
7. Guidelines from the Canadian Association of Pathologists for establishing a telepathology service for anatomic pathology using wholeslide imaging. J Pathol Inform. 2014 Mar 28; 5:15. doi: 10.4103/2153-3539.129455. eCollection 2014.
8. Vodovnik A. Diagnostic time in digital pathology: A comparative study on 400 cases. J Pathol Inform. 2016; 7: 4. Published online 2016 Jan 29.
9. Ho J, Ahlers SM, Stratman C, et al. Can digital pathology result in cost savings? A financial projection for digital pathology implementation at a large integrated health care organization. J 2 d. Pathol Inform. 2014 Aug 28; 5:33. doi: 10.4103/2153-3539.139714. eCollection 2014.
10. Jara-Lazaro AR, Thamboo TP, Teh M, Tan PH. Digital pathology: exploring its applications in diagnostic surgical pathology practice. Pathology. 2010; 42:512-8.
11. Stathonikos N, Veta M, Huisman A, van Diest PJ. Going fully digital: Perspective of a Dutch academic pathology lab. J Pathol Inform [serial online] 2013 [cited 2016 May 16; 4:15.
12. Thorstenson S1, Molin J2, Lundström C3. Implementation of large-scale routine diagnostics using whole slide imaging in Sweden: Digital pathology experiences 2006-2013. J Pathol Inform. 2014 Mar 28; 5:14. doi: 10.4103/2153-3539.129452. eCollection 2014.
13. Ghaznavi F, Evans A, Madabhushi A, Feldman M. Digital imaging in pathology: whole-slide imaging and beyond. Annu Rev Pathol. 2013 Jan 24; 8:331-59. doi: 10.1146/annurev-pathol-011811-120902. Epub 2012 Nov 15.
14. Navid Farahani, Anil V Parwani, Liron Pantanowitz. Whole slide imaging in pathology: advantages, limitations, and emerging perspectives. Pathology and Laboratory Medicine International. 11 June 2015 Volume 2015:7 Pages 23-33.
15. Rohde GK, Ozolek JA, Parwani AV, Pantanowitz L. Carnegie Mellon University bioimaging day 2014: Challenges and opportunities in digital pathology. J Pathol Inform. 2014 Aug 28; 5:32. doi: 10.4103/2153-3539.139712. eCollection 2014.
16. Navid Farahani, Anil V Parwani, Liron Pantanowitz. Whole slide imaging in pathology: advantages, limitations, and emerging perspectives. Pathology and Laboratory Medicine International. 11 June 2015 Volume 2015:7 Pages 23—33.
17. Williams S1, Henricks WH, Becich MJ, Toscano M, Carter AB; Telepathology for patient care: what am I getting myself into? Adv Anat Pathol 2010; 17:130-149.
18. Chen J, Jiao Y, Lu C, Zhou J, Zhang Z, Zhou C; A nationwide telepathology consultation and quality control program in China: implementation and result analysis. Diagn Pathol. 2014; 10.1186:1746-1596-9.
19. Zhao C, Wu T, Ding X, et al. International telepathology consultation: Three years of experience between the University of Pittsburgh Medical Center and KingMed Diagnostics in China.; J Pathol Inform. 2015; 10.4103: 2153-3539.



CHAPTER 2

How to Go Digital in Pathology

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Whitepaper 2016

https://thepathologist.com/app-notes/0016/how-to-go-digital-in-pathology-labpon-whitepaper-made-possible-by-philips/

PROLOGUE

"How to Go Digital in Pathology"

The microscope was invented and developed by the Dutchmen Zacharias Janssen and Antoni van Leeuwenhoek around the 17th century. They made their discoveries with a microscope that initially had two pieces of ground glass and later only one piece of cut glass. A single lens, after all, was much less affected by distortion and chromatic aberration than a compound microscope. This invention-initiated centuries of light microscopic studies of tissues in health and disease and is still very relevant for today's health care. Microscopy is an irreplaceable tool for the pathologist in order to study the architecture of tissues and cells during diagnostics.

The microscope has a longer history with more phases than digital diagnostics. Its digitization is only one aspect of the field of pathology since this field revolves around more than the microscope. For example, the digitization and implementation of whole slide images (WSI) have been going on for years. This effort initiated by academic centres where they investigated its use for research and education and later for multidisciplinary meetings. Digitization will unlock new possibilities for both pathologists and researchers alike, with the use of image recognition and smart software that will help them to work more efficiently, increase accuracy and provides new insights. By embracing technology, the world of digital microscopy can become what the galaxy is for an astronaut. I believe that we are witnessing a turning point and that the time has come to fully integrate digital diagnostics.

At LabPON, we started using WSI in 2010 and discovered right away that we needed to have a fully digital diagnostics workflow to take advantage of all the benefits. As the first laboratory worldwide that succeeded in digitizing the entire workflow, we realize that there are still challenges ahead of us. This huge innovation is not only beneficial for our institute, but for the whole field. To make the most of our experience, we want to share our insights with every department of pathology worldwide. In the Netherlands, I experience an open atmosphere and a willingness to collaborate, which I find to be one of the most important reasons why pathology here is in the vanguard in our field. I am proud to be part of this innovation together with my colleagues.

1. INTRODUCTION

Digital microscopy (pathology) is gaining interest worldwide. For instance, in Scandinavia [1] [2], and in Canada - through the Canadian Association of Pathologists - there are now established guidelines for telepathology service for anatomic pathology [3]. Some Dutch pathology laboratories in the Netherlands are leaders in the transition to work digitally. Laboratory for Pathology East Netherlands (LabPON) is one of the first in the world to make the step to implement a fully digitized histopathological clinical diagnostics workflow.

In this paper, we provide insight into the transition to a fully digitized histopathological clinical workflow in a pathology laboratory. We believe our experience can help other laboratories complete this process smoothly and as cost-effectively as possible.

We describe our transition in the following terms: business vision, logistics, technical aspects, and ergonomics. The implementation in our laboratory was handled gradually and effectively.

About LabPON

LabPON is one of the largest pathology laboratories in The Netherlands and in Europe. It is an independent laboratory with 115 employees of whom 18 are pathologists, handling more than 55,000 histological requests per year, with a turnaround time of 3 to 5 working days. The mission statement of LabPON is to provide fast, efficient pathology service of the highest quality to physicians and hospitals. We realize this is thanks to our clear corporate vision that allows creativity to bloom without interfering with company operations.

2. THE FORMULATION OF THE BUSINESS CASE

General recommendation

At the time of writing, LabPON is working on a detailed flow analysis that can be used for the formulation of a business case (BC). We experienced that it is difficult to compile a basic framework for the BC, but we are convinced that the real advantages only become apparent when the organization has a clear business strategy. Therefore, we believe the most essen-tial part of the business case is gained when a laboratory has a sharp and clear vision in mind: why do we do this and what do we want to achieve.

A preview of a business case

We have made a roadmap (for details see chapter 3), which can be used to calculate your costs. The following list is a preview of its contents, showing that "time and quality" is one of the most important gains:

1. Ceasing internal and external distribution of physical slides (Details in Chapter 7 "Working from a distance," page 14)
2. Optimization of time in preparation and execution of multidisciplinary discussions (Details in Chapter 3 "Implementation and Workflow management," page 7)
3. Digital consultation and revisions (internally and externally) to reduce the change of case mix-ups or loss of slides and reduce the time needed from several days to a few hours (Details in Chapter 7 "Working from a distance," page 14)
4. Multiple applications of digital diagnostics and pathology case viewer. Some examples that are already used in daily practice (Details in Chapter 4 "IT," page 9).
   - 4.1. Total overview of the daily diagnostics, including the size and status of case studies, a simple sorting function and an instant view of the tissue section
   - 4.2. Measurements and counting on the section (Details in Chapter 8 "New developments," page 16).
   - 4.3. Parallel viewing when sections can be digitally placed side by side, or superimposed on each other
5. Image analysis is under development, and we expect that these enhancements to help us diagnose more efficiently, more quickly and qualitatively better (Details in chapter 8, New developments, page 16).

Henk van der Veen, CEO of LabPON, about the cost-effectiveness of working fully digitally

"The revenue model is not based exclusively on acquiring scanners. It is an investment in an entirely different workflow. It is far more profitable to have our diagnostics fully digitally, because the whole process will be transparent, and it streamlines the infrastructure. The real revenue lies in the increase in efficiency. It will make pathology better compatible with external and consultancy services. This larger scale will result in a much better quality, and it will be a vital link for the sub-specializations within pathology as well. It will transform the market."

Figure 1. Shortlist: "How LabPON went digital according to management"

Cost-effectiveness in practice

A pathologist at LabPON about the optimization resulting from digital pathology: "Digital diagnostics forces the organization to critically examine, for instance, whether to use special or routine staining, and to take a closer look at the efficient processing of sections. Take, as an example, the number of lymph nodes that need to be removed in a bowel resection. When there has been a lot of unnecessary cutting done by the laboratory assistant, the pathologist will need to spend more time in microscopy, use unnecessary resources and perform redundant actions. Partly due to the desire to reduce storage capacity, we have critically examined the required number of sections per slide. For most cases, one or two sections per slide will be sufficient. This is just one example of what the critical examination of your process can mean to your organization and how this pushes you towards standardization in every aspect of the workflow."

Digital analysis of the current software system

Van der Veen: "We are pioneers of the movement towards a fully digital workflow in pathology, which we believe to be irreversible. Early on, we recognized that it was not going to be just about buying a scanner. I noticed that Philips is a company that has always been willing to invest in the development of software. We started with a digital analysis of our current software system. This provided insights that formed a powerful basis for our revenue model. This was necessary because the whole infrastructure will need to be revised, and it will be difficult to justify the investments necessary for this transition. It is not simply a matter of replacing existing equipment; it requires a total new workflow. To complete this transition, you will need to have an understanding of strategic matters as well as confidence." Van der Veen has interwoven this progressive view with his business philosophy, propelling pathology forward with entrepreneurial spirit.

Prevention of claims

Digitizing the diagnostic process will greatly reduce the chances of "mix-ups" with patient data caused by material or data handling. At LabPON we integrated a pathology case viewer, IMS and LIMS. It prevents errors that can occur when for instance the tissue image of one patient is assessed in the case viewer and incorrectly reported to another patient in the LIMS. Studies in the US show that pathologists are likely to receive claims [4] and that specimen "mix-ups" are the number one cause of system errors. [5] Furthermore, they have little faith in their in-house system to deal with such errors adequately. [6] Given the high overhead costs related to these claims, the benefits from decreasing handling errors should also be considered for the business case. LabPON advises laboratories that have no experience in this matter to keep in mind that one claim can lead to a significant increase in their liability insurance premium. Considering that almost all pathologists have been involved with errors in various steps of the diagnostic process, [6] full digitization of the diagnostic process will likely lead to a major reduction of the risks of system errors.

We recommend thinking carefully about the transitional path and what your applications will be. By doing this, you will ensure that the transitional process will take up the shortest time possible and it will cost you less as well.

An overview of the different applications

1. **Full digitization of the workflow:** LabPON has clearly defined their applications in advance: consultation, specialization, education, intraoperative consultation, and the ultimate objective of the transition to fully digital diagnostics for histology. Because LabPON has aimed to work fully digitally, they will reach their return on investment sooner.
2. **Consultation:** Digitization enables that subspecialties can develop faster on four levels: internal, regional, national, and international.
3. **The integration of diagnostics into the cancer treatment chain:** This strengthens the position of the pathologist since diagnostic information is accessible to other areas. As a result, the pathologist can closely collaborate with, among others, the radiologist, microbiologist, clinical chemist, and medical geneticist. Big data can be shared very easily in this manner.
4. **Image recognition systems:** Full implementation of the digital workflow in pathology allows image recognition to link data, with the aim to improve the workflow, and ultimately will help to improve the diagnostic process, by making it more efficient, faster, and of a better quality.

3. IMPLEMENTATION AND WORKFLOW

Using a project-based approach, LabPON started the implementation of digital pathology in 2010. Though this was a labour-intensive process, the management team has kept all collaborating parties engaged. Leadership and well-organized change management have been the driving force behind the success of this project. We advise other laboratories to make a workflow analysis at the beginning of the transition and after implementation, because then you can visualize the real workflow optimization. To measure is to know.

Roadmap "How to start"

Below you can find a list of recommended steps when implementing a digital workflow. Taking these steps in this order can determine the success of your implementation. It is very important that all necessary IT adjustments have been tested and improved before purchasing a scanner. When the scanner has been bought, it is important to screen and optimize the software of the pathology case viewer. To prevent repetitive strain injury (RSI) and improve the user-friendliness and interface, we advise to address these issues from the start of the process. Monitors should be set up for the individual user. Ensuring the internal basis for the user is rock solid before setting up the external lines will greatly benefit the infrastructure.

Roadmap how to start

1. Make a workflow analysis of your laboratory
2. Develop a good business plan and an action plan
3. Optimize the IT system (internal and external network, bandwidth, switches)
4. Purchase scanner (technical, image quality, interfaces with LIMS)
5. Screen and improve pathology case viewer
6. Set up interfaces (touchpad, trackball, short key)
7. Set up monitors
8. Install distribution software
9. Set up external network including consultation and revision
10. Set up remote work
11. Set up frozen section diagnostics
12. Develop and use image analysis software

Workflow analyses leads to a faster workflow

A recent study shows that digital pathology may delay the diagnostic process. [7] LabPON sees digitization as an addition to microscopy. In 2013, we investigated the way in which digital diagnostics can actually lead to a faster workflow and higher turnaround time using flow analysis. Our analysis shows that experienced pathologists diagnose just as quickly with both methods, and that inexperienced pathologists are analogously slightly faster. We only examined the diagnostics time and disregarded the logistics of the workflow. Other studies show the same results: the duration of a digital diagnosis is very similar to that obtained by traditional microscopy. [8] Therefore, when we take the whole logistical process into account, digital diagnostics is faster. Another investigation at LabPON shows that working with multidisciplinary team meetings (MDT) in digital diagnostics instead of with physical slides, results in saving 28 hours of administrative work. This is a gain of about 0.7 FTE administrative position.

Since we can compare the workflow from 2013 with the current workflow, the increase is measurable and further improving. This improvement is partly due to optimization of pathology case viewer tools. The accessibility of the files include also a great improvement of the workflow. Functionalities such as measurements, counts and parallel viewing support this.

The technical workflow versus the virtual one

Digitizing the diagnostics workflow begins with a clear concept of how this should be undertaken. This can only be done if the workflow has been defined from receipt of material to the final report.

Traditionally at LabPON, there was a peak in the amount of physical sections for the pathologist in the morning, followed by quieter periods in the afternoon. Although nothing has changed for the cutting of the sections, this is no longer the case in the new workflow. Technical and logistical improvements in LabPON have enabled a more steady, continuous flow with

fewer peaks. To adapt our faster and more efficient technical workflow even further, LabPON has purchased processors that are able to process tissue during the day.

Digital slides are routed continuously to the pathologists, according to the distribution of the WSI. They see straightaway the new additions to their daily worklist and decide to handle it immediately or to do it later. Pathologists are able organize their time more efficiently. In addition, they no longer need to work in the laboratory, as the diagnostics can be performed anywhere, including from home. Working from home allows them to only concentrate on diagnosis and enables them to work faster, more flexible and more efficiently. [9] [10]

Every day, two specially trained technicians and a maximum of three pathologists in LabPON do the grossing. LabPON has the ambition to train these specialized technicians to gross all the sections under supervision. Nowadays, the pathologists dissect most of the larger oncological specimen themselves, which is common practice in most pathology laboratories in The Netherlands.

Nostalgia and a new world

In 2012, in addition to working with the conventional microscope, the pathologists at LabPON were given the option of working digitally with the aim of eventually going fully digital. Studies have shown that the attitude of the pathologist towardsthe digital workflow is important. [11] [12] The duration of the transition varied from three to eight months, depending on the pathologist. The most difficult part was to learn to trust the digital image. Letting go of the traditional way is not only a technical experience, but also a nostalgic one. Since the microscope is a symbol of the pathologist, they might have found it difficult to let go. Though the old way was good, the new way has no principal boundaries. Over time, the number of new applications will increase and it will become clear what this innovation will mean to the profession. Wistful feelings will give way to enthusiasm for this way of working, since its benefits clearly outweighs those of the conventional way of working. A new world is on the rise.

Why not to push the staff to go fully digital

At LabPON the management set a date when all pathologists would ideally work fully digitally. LabPON recommend that other laboratories make the transition gradually and as smoothly as possible, without the pressure that a deadline may cause. The big difference for LabPON was that - as a pioneer - our process is evolutionary. We tried to work with a deadline, but it did not work for the best. Therefore, we do not advise this. Instead, laboratories should pay attention to having a good training program and review all your digital applications.

We also advise shortening the time of the transition as much as possible by offering a good training program. The shortest possible transitional time also reduces expenses.

Summarized:

1. Approach pathologists who are enthusiastic early adopters
2. Involve and coach them in drawing up a training program

3. Make sure they all receive proper training in digital applications to maximize the benefits and improve the efficiency of their work
4. Implement the process organically and organize recurring digital viewing sessions with the participants

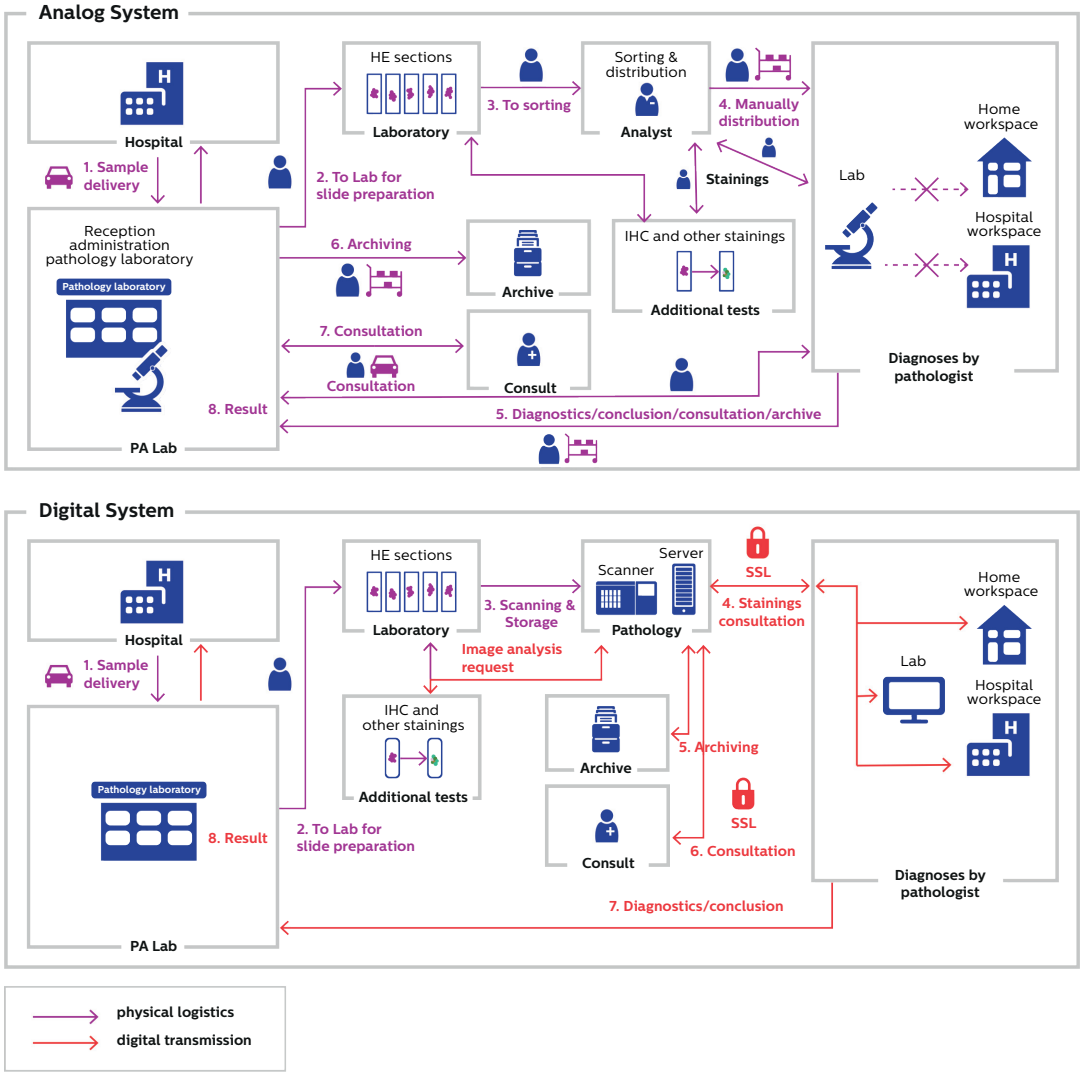


Figure 2. Pathology workflow for histology cases

4. IT

Philips has used its experiences with comparable projects in radiology for the IT development in pathology. Together with LabPON, they worked out the digitization and implementation. The developers learned a lot from their dedication to this project, and it improved their ability to solve problems. Essential to this new system was the software development and implementation. Helped by their move to a new building in 2012, LabPON had the unique opportunity to critically asses the structure of their software system. Nowadays, LabPON offers WSI via a server without delays or interruptions, and stores many GBs of data simultaneously. Pathologists are able to retrieve WSI from this server easily.

The standard digital connections are essential to the safety and efficiency of the pathology case viewer, image management system (IMS) and the Laboratory Information Management System (LIMS). Based on experiences, these connections were adjusted and improved each month.

The on-screen worklist offers a very good overview for his diagnostic work.

Pathologist case viewer

The case viewer offers new possibilities, allowing the pathologists to see at a glance the diagnostic cases scheduled for that day. They can see the daily list of tissue numbers, section numbers and medical service requests, and sort this list by preference. The software keeps track of what the pathologist has seen per case study and per section.

Case viewer and the connection with a Laboratory Information Management System (LIMS)

Dr. Nina Kooij, Pathologist at LabPON: "A full, bidirectional communication between pathology case viewer and LIMS is necessary to complete the digital diagnosis process. Viewing digital slides, reporting a case in the LIMS, and requesting additional stains need to be part of a logical and fully linked system that has to be acceptable for the pathologist reading the case. It also has to prevent case mix-ups.

To make sure that we can efficiently and safely read and sign out a case, we are in the process of developing a fully connected system that synchronizes in both systems important aspects, like changing the case viewed and assigning a responsible pathologist, resident or technician.

Scanning of the slides is executed directly after the staining and drying process steps. The case can be assigned to a case holder/ pathologist in either case viewer or LIMS, depending on the preference of the laboratory. This will also change the case holder in the linked system. In the future, we hope to have developed a routine for assigning cases automatically, making it possible to assign cases while they are being scanned.

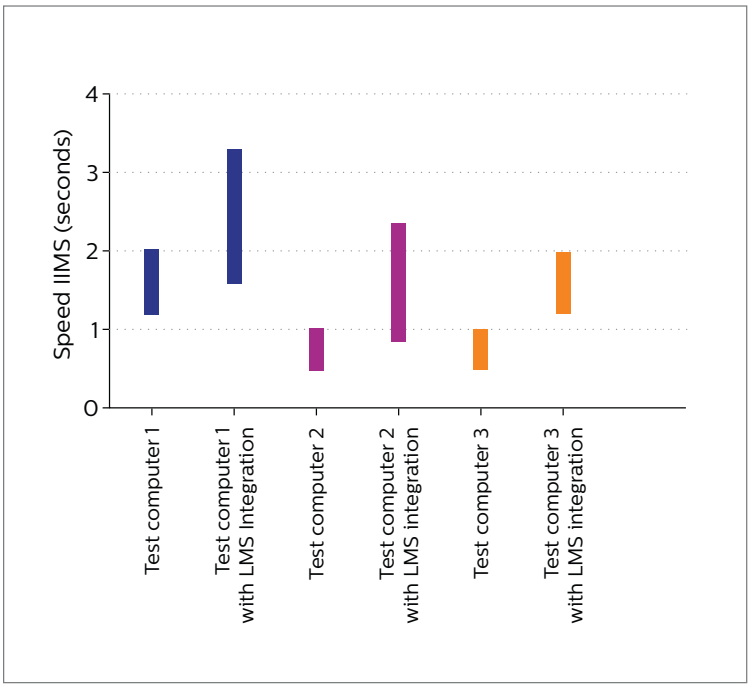
The case is placed in the folder 'In preparation' as soon as the first slide is scanned. The total number of expected slides will be sent by the LIMS in response to the request for information after the first slide is scanned. This requires an option in the LIMS to indicate whether a staining is suited for scanning, and to set an accurate number for the expected amount of slides. As soon as all slides are scanned, the case is moved to the folder 'For review' and ready for the pathologist to read and report.

Requesting an additional stain should move the case to an additional folder in both systems to indicate that the case is once again incomplete. When the additional stains are scanned, the case will be returned to 'For review'. Signing out will move the case to 'Finished' and will prevent any changes by the pathologist who signed the case in both systems, unless the case will be reopened by an authorized person. Changing a case in either pathology case viewer or LIMS will cause the other system to follow, if the case is available, or to go to a blank screen if there is no matching case found. A speech recognition system will advise the user to save or delete the case, or to finish the current case.

It is essential to have both systems fully linked. This will prevent mix-ups, but it will also make the system more convenient for the pathologist."

Test Computers			
All computers were equipped with operating system Windows 7			
	(Test computer 1)	**(Test computer 2)**	**(Test computer 3)**
Processor	Core2Duo E8400 3.0 Ghz	Core i5-650 3.2 Ghz	Precision T1700 SFFXQC E3-1241 V3 3.5 Ghz
Memory	4 GB	4 GB	2 X 4 Go DDR3-1600
Hard drive	120 GB SSD	120 GB SSD	256 GB SSD
Video card	Matrox M9128 LP	Matrox M9128 LP	Nividia QUDRO K620

Table 1. Specifications of the computers used in the test



Graphic 1. Speed image rendering in seconds for 3 computers

Storage policy and Security

Policy of LabPON

LabPON is a diagnostics laboratory, which means that most of its archived images will not be used after 6 to 8 weeks. We have therefore chosen to store the digital images accordingly and to remove them after this period. The archived glass slides are stored for up to 30 years under normal guidelines.

Since the Dutch Pathology Association is discussing legal regulations for digital pathology, there is no legal framework for image storage. However, it is permitted in the Netherlands to make a diagnosis based only on digital images. [17]

Why archiving all slides will be required?

Archiving WSI will make it easier and more efficient for you to prepare for multidisciplinary team meetings (MDT) because:

1. Comparisons with previous diagnoses (revisions) are easy to retrieve
2. Reviews can be viewed simultaneously. Everyone is looking at exactly the same image
3. It saves time by eliminating archiving work
4. The quality of WSI remains constant contrary to glass sections, which are fragile and can fade
5. It also covers research and education purposes

The present and the future

LabPON has a server with a capacity of 89 TB, which is enough for about 2.5 months. Storing our entire WSI would require about 450 TB per year. We believe that in an ideal world all files should be stored at two different locations. This would ask for a storage capacity of 900 TB per year, based on the average size of a scanned tissue sections at LabPON. The necessary storage capacity depends on the scanner type and the workflow. For the latter, it is important to consider the way the sections are scanned (40x or 20x), what kind (mix) of material they have (are there many biopsies or resections?), and what kind of protocols are applied when cutting the sections; for example, are there many sections for each case and/or large tissue sample on cassette? 3-D scanning can also greatly affect the needed storage capacity. As a result, the space for the same number of cases can differ greatly per year and per laboratory.

To reduce the required storage space, Philips is developing smart storage software, which allows the WSI to be stored more compactly, while keeping the quality of the image intact. Many reliable solutions have been devised to guarantee the security of the server. The current focus is on dealing with the file size, leading to possible cost reduction in storage, while speed and quality of the server are constantly being improved.

Reasons for LabPON to delete images after 6 to 8 weeks

Image storage is one of the main bottlenecks. At this moment, LabPON cannot afford to store all their WSI permanently. We are waiting for new developments that will make storage more cost-effectively, e.g. by compressing images. This position is similar to that of other (non-academic) pathology laboratories in The Netherlands that diagnose partly with WSI. Some academic centres, such as the University Medical Centre in Utrecht, store all their WSI permanently, but they archive their glass slides at the same time. These physical slides are used for study and research.

5. SCANNER

A whole slide scanner is essentially a computer-controlled microscope that is attached to a camera with advanced sensors. Some scanners have pre-focusing functionalities. The following are the general components of a scanner. [11]

1. A microscope with lens objective
2. A light source (bright field and/or fluorescent)
3. Robotics to manoeuvre the physical slide swiftly inside the machine without breaking the slide
4. Digital cameras to capture the WSI
5. Computer hardware
6. Software for a fast and secure digital infrastructure and management, and for visualization of the WSI

Choice of scanner

There are different kinds of scanners. Before you buy one, you should decide what kind of scanner you need. They can be used for research, education, consultation, and diagnostics. There are several important components to consider, and the target is a very important one. You should look for a scanner that is easy to operate, provides good image quality, has high throughput with low re-scan rate and is affordable.

LabPON tested scanners from different venders for a longer period of time. During our tests, we noticed that there is room for improvement when it comes to 3-D scanning, image quality, sections, improving areas that are out of focus, and more.

Points to consider when purchasing a scanner:

1. Image quality (the higher the quality the more details you will see)
2. Speed of scanning
3. Size of the files (see our chapter on storage on page 11)
4. User-friendliness
5. 3-D scanning option
6. Option to scan big slides
7. Option to scan fluorescence
8. Option to do automated quality control and automated rescan

Software	Usability	Image quality	File size	Speed	Viewer
Good	Good	Moderate / Good	800 MB to 1500 MB	Good (± 2.5 min)	Moderate
Moderate	Moderate	Poor / Moderate	500 MB to 1 TB	Moderate (± 3.5 min)	Moderate

Table 2. Digital pathology system evaluation

Choice for the Ultra-Fast Scanner

LabPON choose for the Philips IntelliSite pathology solution because of the high quality of its technology and its ease of use. Limitations like lack of 3-D scanning, scanning big slides and fluorescence did not play a big role.

The use of the scanner in the diagnostic workflow

LabPON have five high-throughput Ultra-Fast Scanners (UFS). The technicians usually run four scanners simultaneously. Most of the daily turnover of slides are scanned at night and istributed in the morning to the available pathologists. However, because of the continuous, nonstop workflow, the technicians also use the scanners during the day and divide the slides equally among the scanners.

- Racks are used simultaneously with a total capacity of 300 slides
- Average scanning time in LabPON is +/- 2.5 minutes per slides; +/- 50 minutes for one rack of 20 slides

- During the peak times there is more than 400 MB of data transferred from the scanners to server each second

Validation scanner

The objective of the validation was twofold: are the WSI good enough in comparison with a light microscopic image, and are the technical aspects of digital and diagnose critically assessed?

The initial validation of a scanner was done with 173 diagnostic case comprising 1042 slides. The normal composition of the day of this routine diagnostic laboratory is used as standard. In this validation LabPON has not specified the differences in scanning time in relation to the average size of the slide deliberately. These cases were digitally diagnosed by a panel of three pathologists. After three days, the panel diagnosed the exact same material through a light microscope.

At LabPON there are on average 800 slides produced per day. If 0.4% cannot be diagnosed digitally after scanning, it would mean that three slides should be re-scanned per day. We found no obvious problems in diagnosing WSI. The daily number of slides that need to be re-scanned is deemed to be acceptable (an average of three per day). We concluded that WSI with these scanners is suitable for diagnostics.

Scanner areas for improvement

Occasionally, mechanical problems could occur when grabbing or moving slides with current technology scanners. This is often related to slide related issues, such as sticky, not well dried out, or incorrectly covered slides. The scanner will give an error message and will stop processing. This is an area for improvement for both laboratories, as well as the use of less sensitive technology within the scanner. But there are more challenges to overcome in the area of slide scanning. Scanning 3-D images is still problematic. Specifically long scan times and huge data files generated are a big challenge. 3-D images are important especially in cytology diagnostics. Another improvement opportunities are with the selection of settings for scanners. The reduced sensitivity setting, to increase scanning speed, sometimes causes problems with skipping a small piece of tissue. This can have major negative implications for diagnostics. Especially, light fabrics such as adipose tissue and immunohistochemical stained slides can be difficult to scan.

6. ERGONOMICS

In several studies, ergonomics is named as one of the benefits of working fully digital. [2] [9] [11] The transition from analog to digital diagnosis naturally results in lesser ergonomic problems from working at a microscope, mainly neck and shoulder pain. During the transition to working fully digitally, LabPON monitored the ergonomic aspects of our workflow, and it became clear that unilateral movements with a mouse in particular pose a risk, because of their repetitive

nature, resulting in repetitive strain injury (RSI). Therefore, we looked for ways to vary between interfaces. Our solution for this problem is to use more than one device. In collaboration with Philips, we developed a selection of tools for different devices.

Keyboard shortcuts

Philips made efforts to minimize the number of clicks by developing an efficient user interface which allows the use of keyboard shortcuts. This resulted in user-friendly software that is more intuitive to work with.

User Input Devices

We developed a touchpad tool and a tool for the "Space Navigator" to prevent RSI symptoms. The touchpad works in the same way as a tablet or mobile phone and allows pathologists to choose their user interfaces.

Monitor

At LabPON we looked into the quality aspects of monitors to find the best one for digital diagnostics. We experimented with monitors and found that almost all monitors are good for diagnostics, although we recommend higher quality monitors.

Selecting the right monitor:

1. Use at least two monitors. One for the normal workflow and one that replaces the microscope and is used for viewing the WSI.
2. Use a monitor of 24-30 inches (61-76 cm). In our opinion, the best size is 27 inches. The screen should not be too small so you can still see all the details. The screens should also not be too big, otherwise the pathologist will risk too much head movements
3. Use two monitors of the same size and the same models. This is important because different monitors have different letter sizes and different colours, even after calibration.

7. WORKING FROM A DISTANCE

Digitization creates new applications and possibilities for performing diagnostics from a distance, making it even possible to perform them from outside the organization. Working from a distance is a big benefit of digital pathology. [10] [11] Here we discuss several internal and external applications of pathology from a distance, also called telepathology. Once a pathology lab workflow is fully digitally, regional and national digital networks and platforms can be set up, which can improve the diagnostics. LabPON is setting up a regional network in collaboration with the University Medical Center Groningen (UMCG) and Hospital Isala in Zwolle. Also, international consultations are digitally much simpler and better accessible. The biggest challenge of external consultations is security in transmission of patient information. With the use of digital pathology and its ability to stream images

(WSI) digitally for external consultations, it has become very topical to send patient information securely to other laboratories. For this several options have been identified. Recent developments will soon ensure that external consultations can be done, within a very short time, digitally.

Internal consultations

At LabPON we have an internal digital network for consultations. Thanks to technical and logistical innovations these consultations are more widely accessible. Moreover, subspecialties are easier to plan and maintain, knowing that there is a minimum number of cases a pathologist must view for their subspecialty.

Remote diagnostics

Being able to work from a distance has many advantages. For planning and organizing it provides peace of mind knowing that working space will not be an issue. There are currently no official regulations for remote diagnostics, but LabPON advises using the same technology as in the main workplace.

A few examples of the benefits of working externally:

1. If the pathologist cannot go to work, perhaps due to a sick child, poor weather conditions or traffic issues, he can still work from home.
2. When a pathologist has an external meeting, conference or appointment that takes up part of the working day, she can still use the remaining time to work from a distance and can be consulted for specialized cases.
3. Part-time pathologists have the possibility of completing histopathology cases outside the laboratory after office hours without having to ask a colleague for assistance.

The security of the digital connections is guaranteed by Citrix connections. For outside work we use a dedicated Virtual Private Network (VPN).

Frozen section services

LabPON provides intraoperative services for several hospitals. We want to use remote diagnostics for this as well in the future.

Currently the pathologist and the technician who cut the frozen section have to travel to, in our situation, an Operating Room (OR) in another hospital. If the surgery takes longer than expected, as is often the case, the pathologist may have to wait for several hours. Digital pathology can potentially smoothen this procedure in the future. We envision that the technician could, with the on-line aid of a pathologist, perform the technical part on-site, scan the slides digitally and send the WSI to the patholo- gist. Then the pathologist can evaluate the case behind his/her desk and even easily consult a colleague if necessary, while working in the leisure of his/her own workplace. This digital workflow thereby guarantees a better, more cost-effective diagnosis, since traveling and waiting time could be reduced.

However, this is not yet implemented in our diagnostic workflow.

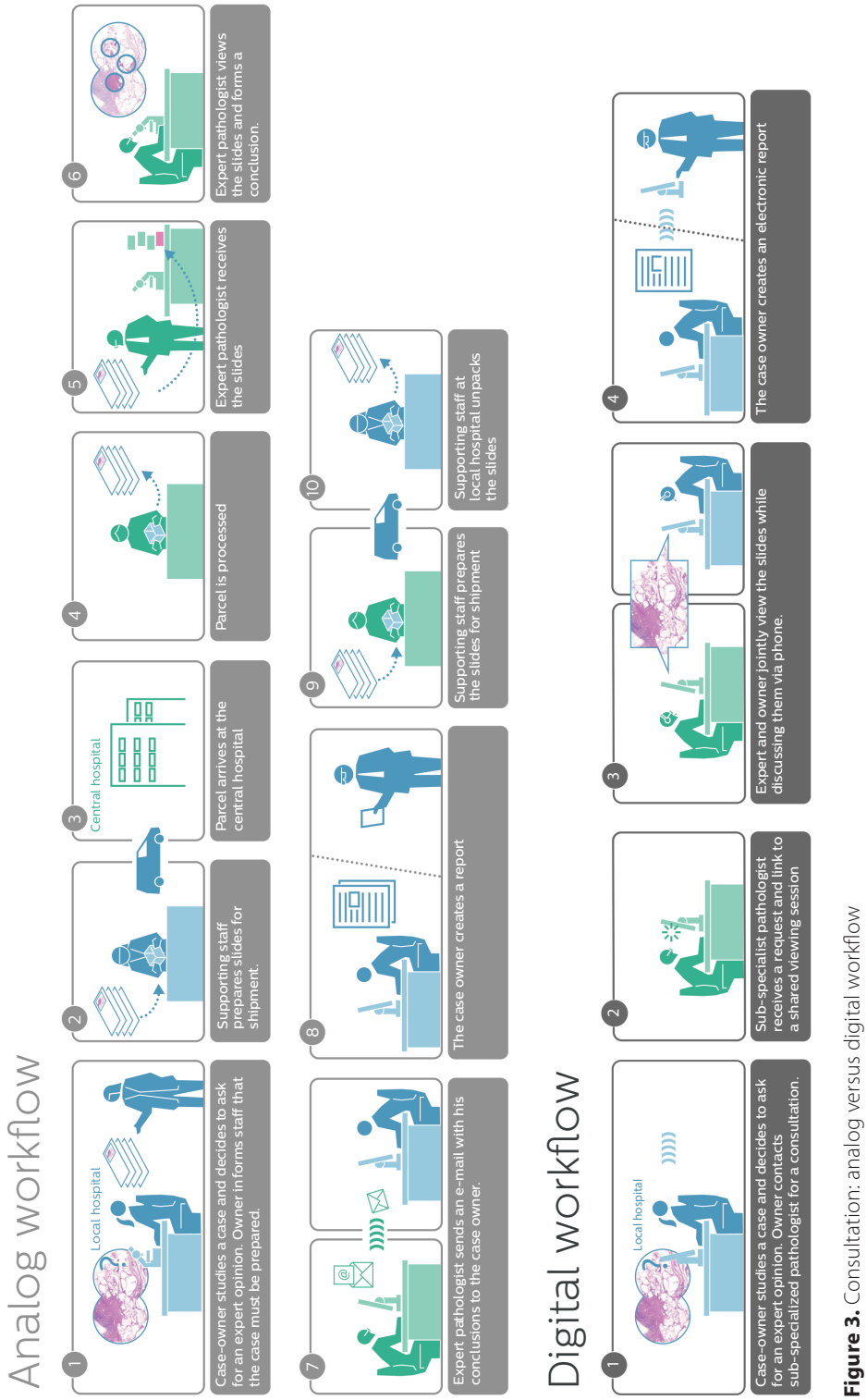


Figure 3. Consultation: analog versus digital workflow

External services

LabPON is a non-profit organization and we support other laboratories when they are temporarily understaffed. Thanks to the digitization of our workflow, it is more efficient, allowing us to provide more of these services to external partners. [13]

Developing a network of pathologists

Together with the hospital Isala in Zwolle and the UMCG, we are working on a digital network that will connect their pathologists as well. We notice that our team of pathologists are consulting their colleagues in this network more frequently.

Regional digital network

Dr. Jos Bart, pathologist at the hospital Isala in Zwolle: "At the moment we are working together with LabPON and the UMCG on creating access for all of the almost 50 pathologists to patient data and corresponding microscopic images on one central server. This regional network could be expanded into larger networks, and we are starting with the three institutes. Philips has a remarkable amount of knowledge and is very skilled in the software that is part of the infrastructure of digital microscopy, much more so than other suppliers, and we benefit gladly from their expertise."

Pathology image exchange project

The Dutch Society for Pathology (NVVP) has created a working group to set up a national platform that will enable laboratories to exchange their WSI. This project, called PIE (Pathology Image Exchange), is affiliated with the PALGA Foundation. PALGA is the Dutch national database where all reports generated by all Pathology departments (academic and non-academic) are stored, as well as a network for data exchange for all pathology laboratories in The Netherlands. Every laboratory in The Netherlands has been approached to be a part of this project.

Working group member Professor Paul Van Diest of the University Medical Center Utrecht (UMCU) about the ambitions of this national initiative: "Many consortia are setting up local digital networks at this time, but it is extremely complex to connect everything. What this working group is doing, in parallel to these local initiatives, is developing a national image exchange platform for all to connect."

Professor Philip Kluin of the UMCG: "In 2008 we started projects related to external networks at the UMCG, which involved LabPON and hospital Isala in Zwolle, but also the Martini Hospital in Groningen. We turned our focus quickly to the digital connection with and the secure integration of PALGA where all patient data is managed since this is necessary for returning a regular report and claiming a consultation digitally. To avoid confusion and delay a pathology report must be linked safely and directly to the corresponding digital images. It takes times and a good collaboration of the different IT departments to implement a flawless consultation system within the region and the country. Establishing a central server that will connect LabPON, Isala

and UMCG is definitely a good idea, but it is also a challenge to integrate the necessary patient data on one server. That is why I am very happy with the national initiative PIE and the way PALGA is managing and controlling the exchange of patient data. Digital pathology will improve collaboration between the centers, both regionally and nationally."

8. NEW DEVELOPMENTS

The techniques behind digital pathology are not new, but important steps in the IT have been made allowing for a faster progress. In this chapter, Alexi Baidoshvili illustrates current refinements and gives insights into future developments in pathology.

Faculty of Technical Medicine, Technical University of Twente

LabPON collaborates with several partners, including the faculty of Technical Medicine at the Technical University of Twente. This faculty offers strong technical training but also has a very extensive medical program. Nowadays the technical professionals think along with the medical specialists making it more likely to come up with suitable solutions.

Image analysis

Pathologists have to quantify often. Any help in this area is highly appreciated. Software can do this work more precisely and more efficiently. This sort of assistance is akin to having someone who handles the preparatory work. For example, if a pathologist marked in the LIMS the cassette that contains the tumor during cutting a section, the software can automatically grade the tumor and perform other quantitative calculations. A good image recognition program can locate and grade the tumor as well. This will save a lot of time, for example in the assessment of a prostatectomy. [14] [15] We want to collaborate with the Radboud University Medical Center in Nijmegen, the UMC Utrecht, UMC Groningen and the University of Twente to further develop this software. This is one example of an important tool that will resolve qualitative and quantitative problems and may help to improve the diagnostics.

Image recognition techniques for HE staining

Jeroen van der Laak, Research Group Leader in pathology at the Radboud University Medical Center in Nijmegen, is working with a group of eight researchers on further developing an image recognition system. The group focuses on the content of image recognition techniques for HE staining in the diagnostic process.

[16] Jeroen van der Laak agrees with Alexi Baidoshvili that digitization of pathology is one cornerstone of the profession's progress. The implementation of image recognition techniques will refine the work of the pathologist.

Figure 4. PhilPath software for annotation tumor tissue on standard H&E stained digital slides. It is used for research purposes and not in diagnostic procedures.

Patient treatment plan and prognosis

Digital tumor images allow for a closer examination of the tissue properties. [17] This is one of the latest techniques whereby tissue properties that cannot be distinguished with the naked eye are digitally regrouped. The software classifies the tumor according to several characteristics. This method is called "Deep Learning". The survival rate of tumors related to its physical properties is subject of scientific research. Tumor tissue could be graded differently in the future. This could improve the grading of tumor tissue, which will result in more accurate patient treatment plans and prognosis by the oncologist.

'Slide' tracking

In the case of prostate diagnostics, several biopsies are usually taken from different regions of the prostate. These biopsies may be fragmented, and, as a result, part of the tissue can easily be missed under the microscope. Using WSI will prevent this be-cause the software records and marks all areas that have not been screened. The pathologist will not only see the scanned image on the monitor but also this information. Furthermore, if a pathologist is interrupted while viewing a specimen, he will not have to start all over again when using WSI, whereas with a conventional microscopy he would still have to. Therefore, the slide tracking feature of the software toolset will save a great deal of time and prevent errors.

The "3-D scanning" for cytology and histology

A histological specimen can be scanned reliably in two dimensions, but cytological specimens and some histological specimen must be scanned in three dimensions in order to be considered sufficiently reliable. Three-dimensional scanning, however, is time consuming. Data storage requires a lot of memory. Philips is developing a technique to scan multiple layers at the same time. They also have a new technique, the "smart compression method," which reduces the necessary memory storage used. It preserves the main image on which the diagnosis is established optimally and compresses the part that was screened briefly. LabPON is going to test this further.

Molecular pathology

In the field of molecular pathology there is a method being developed that allows for malignant lesions in slides to be marked digitally for genetic research. The marked malignant lesion will be punched out automatically by a robot for making tissue arrays or molecular analysis. This development has only just begun, but it shows us what possibilities there are.

Epilogue

The team at LabPON walked through the entire process of digitization of diagnostics. In six years we have solved many issues and invested a lot of time and money. We are now fully digital, and we are equipped with all the necessary amenities, but it is still not optimal. Because we went through this process, we gained a lot of experience and insights. Now we can better address our remaining challenges and come up with solutions. At LabPON the entire medical staff, several employees and management are committed to solving the challenges ahead and to keep improving.

Digitization of routine diagnostics in pathology is in early stages at other laboratories. Its implementation is very complex and needs to be prepared well, and requires investments. It is very important to formulate a good business vision and a roadmap based on experience instead of assumptions. To this end, we offer our insights for the benefit of other laboratories, and to serve as an example in the digitization of all routine diagnostics. This has always been one of the key drivers of our organization.

GLOSSARY

LabPON	Laboratory for Laboratory for Pathology East Netherlands
IMS	Image Management System, tracking system
Pathology case viewer	Software tool designed to get pathologists through cases as fast as possible, and having easy access to information and resources to for informed decision making.
LIMS	Laboratory Information Management System
MDT	Multidisciplinary team meeting
NVVP	The Dutch Society of Pathology
PIE	Pathology Image Exchange, a Dutch initiative from the NVVP to develop a national image exchange platform
PALGA	The nationwide network and registry of histopathology and cytopathology in the Netherlands
UDPS	Universal Decentralized PALGA System
UFS	Philips IntelliSite Ultra-Fast Scanner
UMCG	University Medical Center Groningen
UMCU	University Medical Center Utrecht
VPN	Virtual Private Network
WSI	Whole Slide Image

REFERENCES

1 Lundström C, Thorstenson S, Waltersson M, Persson A, Treanor D. Summary of 2nd Nordic symposium on digital pathology. J Pathol Inform 2015;6:5.

2 Thorstenson S1, Molin J2, Lundström C3. Implementation of large-scale routine diagnostics using whole slide imaging in Sweden: Digital pathology experiences 2006-2013. J Pathol Inform. 2014 Mar 28;5:14. doi: 10.4103/2153-3539.129452. eCollection 2014.

3 Guidelines from the Canadian Association of Pathologists for establishing a telepathology service for anatomic pathology using whole- slide imaging. J Pathol Inform. 2014 Mar 28;5:15. doi: 10.4103/2153-3539.129455. eCollection 2014.

4 David M. Studdert, LL.B., Sc.D., M.P.H., Michelle M. Mello, J.D., Ph.D., M.Phil., Atul A. Gawande, M.D., M.P.H., Tejal K. Gandhi, M.D., M.P.H., Allen Kachalia, M.D., J.D., Catherine Yoon, M.S., Ann Louise Puopolo, B.S.N., R.N., and Troyen A. Brennan, M.D., J.D., M.P.H. Claims, Errors, and Compensation Payments in Medical Malpractice Litigation. N Engl J Med 2006; 354:2024-2033May 11, 2006DOI: 10.1056/ NEJMsa054479

5 David B. Troxel (2006) Medicolegal Aspects of Error in Pathology. Archives of Pathology & Laboratory Medicine: May 2006, Vol. 130, No. 5, pp. 617-619.

6 Suzanne M. Dintzis MD, PhD, Galina Y. Stetsenko MD, Colleen M. Sitlani MS, Ann M. Gronowski PhD, Michael L. Astion MD, PhD, Thomas H. Gallagher MD. Communicating Pathology and Laboratory Errors Anatomic Pathologists' and Laboratory Medical Directors' Attitudes and Experiences. DOI: http://dx.doi.org/10.1309/AJCPJF1YUFG6GTFI 760-765 First published online: 1 May 2011

7 Rebecca Randell, Roy A. Ruddle, Darren Treanor – Barriers and facilitators to the introduction of digital pathology for diagnostic work – MEDINFO 2015 eHealth – enabled Health

8 Farahani N, Pantanowitz L. Overview of Telepathology. Surg Pathol Clin. 2015 Jun;8(2):223-31. doi: 10.1016/j.path.2015.02.018. Epub 2015 Apr 4.

9 Ghaznavi F, Evans A, Madabhushi A, Feldman M. Digital imaging in pathology: whole-slide imaging and beyond. Annu Rev Pathol. 2013 Jan 24;8:331-59. doi: 10.1146/annurev-pathol-011811-120902. Epub 2012 Nov 15.

10 Rohde GK, Ozolek JA, Parwani AV, Pantanowitz L. Carnegie Mellon University bioimaging day 2014: Challenges and opportunities in digital pathology. J Pathol Inform. 2014 Aug 28;5:32. doi: 10.4103/2153-3539.139712. eCollection 2014.

11 Navid Farahani, Anil V Parwani, Liron Pantanowitz. Whole slide imaging in pathology: advantages, limitations, and emerging perspectives. Pathology and Laboratory Medicine International. 11 June 2015 Volume 2015:7 Pages 23—33.

12 Randell R, Ruddle RA, Treanor D. Barriers and facilitators to the introduction of digital pathology for diagnostic work. Stud Health Technol Inform. 2015;216:443-7.

13 Mendelson DS, Erickson BJ, Choy G, Image Sharing: Evolving solutions in the age of inter operability – J. Am Coll Radiol 2014; 11 (12 P+B) 1260 -9.

14 Shaimaa Al-Janabi, André Huisman, Geertruida N. Jonges, Fiebo J.W. ten Kate, Roel Goldenschmeding, Paul J. van Diest. Whole slide images for primary diagnostics of urinary system pathology: a feasibility study- Journal of Renal Injury Prevention - dec 2014

15 Philipe Camparo, Lars Egevad, Ferran Algaba, Daniel M. Berney, Liliane Boccon-Gibod, Eva Compérat, Andrew J. Evans, rainer Grobholz, Glen Kristiansen, Cord Langner, Antonio Lopez-Beltran, Rodolfo Montironi, Pedro Oliveira, Ben Vainer, Murali Varma- Utility of whole slide imaging and virtual microscopy in prostate pathology.- ACTA Pathologica microbiologica et immunoclogica scandinavica - april 2012

16 Litjens G, Sánchez CI, Timofeeva N, Hermsen M, Nagtegaal I, Kovacs I, Hulsbergen-van de Kaa C, Bult P, van Ginneken B, van der Laak JAWM. Deep learning as a tool for increased accuracy and efficiency of histopathological diagnosis. Nature Scientific Reports 6: 26286, 2016

17 Hanna MG, Pantanowitz L, Evans AJ. Overview of contemporary guidelines in digital pathology: what is available in 2015 and what still needs to be addressed? J Clin Pathol. 2015 Jul;68(7):499-505. doi: 10.1136/jclinpath-2015-202914. Epub 2015 May 15.

PART 2

GOING DIGITAL

Chapter 3. Validation of a whole-slide images-based teleconsultation network

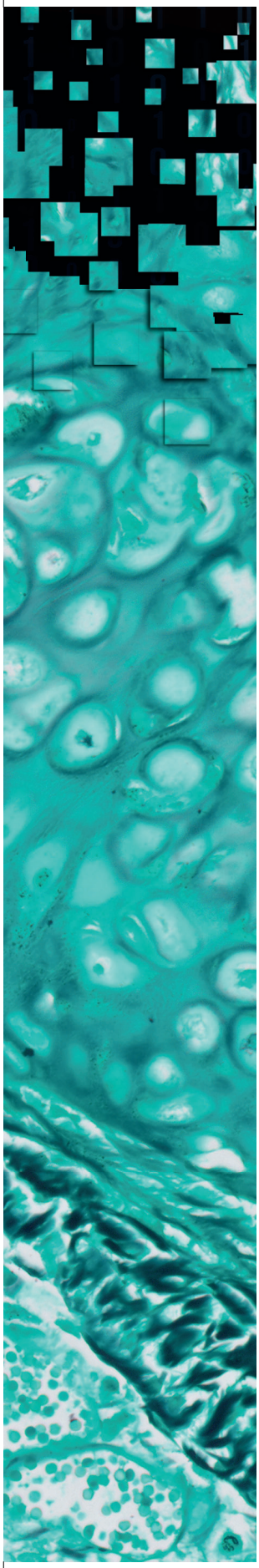
Chapter 4. Evaluating the benefits of digital pathology implementation:

Time savings in laboratory logistics

Chapter 5. A whole-slide imaging based workflow reduces the reading time of pathologists

Chapter 6. Computer aided quantification of intratumoural stroma is an independent prognosticator in rectal cancer

Chapter 7. General discussion



CHAPTER 3

Validation of a whole-slide image-based teleconsultation network

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ABSTRACT

Background: Most validation studies on digital pathology diagnostics have been performed in single institutes. Since rapid consultation of extramural experts is one of the most important use cases for digital pathology laboratory networks, the aim of this study was to validate a whole slide image-based teleconsultation network between three independent laboratories.

Methods: Each laboratory contributed 30 biopsies and / or excisions, adding up to 90 specimens (776 slides) of varying difficulty and covering a wide variety of organs and subspecialties. All slides were centrally scanned at 40x scanning magnification and uploaded, and subsequently digitally assessed by 16 pathologists using the same image management system and viewer. Each laboratory was excluded from digital assessment of their own cases. Concordance rates between the two diagnostic modalities (light microscopic vs digital) were compared. Loading speed of the images, zooming latency and focus quality were scored.

Results: Leaving out 8 minor discrepancies without any clinical significance, the concordance rate between remote digital and original microscopic diagnoses was 97.8%. The two cases with a major discordance (for which the light microscopic diagnoses were deemed to be the better ones) resulted from a different interpretation of diagnostic criteria in one case, and an image quality issue in the other case. Average scores for loading speed of the images, zooming latency and focus quality were 2.37 (on a scale up to 3), 2.39 (scale up to 3) and 3.06 (scale up to 4), respectively.

Conclusions: This validation study demonstrates the suitability of a teleconsultation network for remote digital consultation using whole slide images. Such networks may contribute to faster revision and consultation in pathology while maintaining diagnostic standards.

INTRODUCTION

Where radiology uses digitized images routinely in the diagnostic process for many years now, the use of digitized microscopic slides (so-called whole slide images; WSI) in diagnostic pathology is still limited. Regulatory issues and the high costs associated with scanning slides and storing the digital images still impede large scale introduction of 'digital pathology'. At the same time, most pathologists now recognize the advantages of a fully digitized workflow: increased efficiency and ergonomics, better patient safety and higher quality of diagnostics [1-3].

Remote digital revision and consultation is an important use case of digital pathology and is increasingly used in the USA and a few other countries but has not been widely studied [4-6]. In cases where a patient is referred from one hospital to another for further treatment, the pathology material (glass slides and tissue blocks) is usually shipped by regular mail to the other hospital. In the receiving pathology laboratory, the slides are re-assessed ('revised') by a local pathologist. This entire procedure is cumbersome and easily takes up to two weeks. Similarly, consultation of an expert pathologist for difficult cases may take up to two weeks if glass slides have to be shipped. As a result, the clinical trajectory of the patient is delayed, which is stressful for the patient and potentially even harmful for disease outcome.

We hypothesized that a significant time-gain may be realised if digital pathology networks allow for revision or consultation at the remote site. The aim of this study was to design and evaluate a pilot digital pathology network between three pathology laboratories in the Netherlands. Although a number of validation studies have been carried out comparing WSI with reviewing traditional glass slides at the local pathology centre [7-11], it was deemed necessary to separately validate remote diagnostics in this network before actually using this network for real cases. In this paper, we describe the setup of the network and the validation results on the first 90 cases.

MATERIALS AND METHODS

The network

A teleconsultation network was set up between the Laboratory for Pathology East Netherlands (LabPON; lab H) in Hengelo and the pathology laboratories of the Isala Clinic in Zwolle (lab Z) and the University Medical Center Groningen lab G), The Netherlands. The network used an IntelliSite image management system (IMS) which was installed on a remote server in a datacentre in Amsterdam, The Netherlands (Philips Digital Pathology Solutions, Best, The Netherlands). All three locations were previously tested for Information and Communication Technology (ICT) infrastructure. Thus, the IMS was accessible through the internet, allowing upload and access of data from the three labs with a similar bandwidth and latency. By hosting the server fully separate from the three participating locations, potential firewall restrictions by the private hospital networks were circumvented.

Case selection and logistics

Each lab selected 30 routine cases originally diagnosed between 2012 and 2013 from routine diagnostics, covering varying tissue types to ensure a representative case mix (Table 1).

	Specimen type		
Organ	**Biopsy**	**Resection**	**Total**
Oesophagus	1	0	1
Stomach +/- duodenum	7	0	7
Appendix	0	1	1
Colon	6	1	7
Liver	3	0	3
Vulva	2	1	3
Cervix uteri	3	1	4
Endometrium	5	1	6
Tuba	0	1	1
Ovary	0	3	3
Placenta	0	1	1
Breast	2	3	5
Lymph node	7	3	10
Bone marrow	5	0	5
Skin	9	7	16
Tongue	0	1	1
Lung	0	2	2
Kidney	6	0	6
Urinary bladder	1	0	1
Prostate	3	0	3
Brain	1	0	1
Soft tissue	1	2	3
Bone / cartilage	0	1	1
Total	61	29	90

Table 1. Tissue types from all laboratories.

Essentially, the cases were consecutive but were supplemented by some renal, bone marrow and lymphoma biopsy cases. All available slides including serial sections, histochemical special stains and immunohistochemical stains were scanned and evaluated, except immunofluorescence slides of renal biopsies. Slides were scanned with an IntelliSite ultrafast scanner (UFS; Philips) at 40x (resulting in a specimen level pixel size of 0.25x0.25µm^2) located at LabPON and uploaded to the IMS server. The standard proprietary Philips wavelet based compression was applied, which means that it is perceptually lossless.

For the actual digital pathology, a questionnaire (EXCEL format) was distributed to be completed by the individual participating pathologists. After the distribution of the digital cases

and the questionnaire, the pathology reports containing all necessary clinical and macroscopy data and the original glass slides were sent by post. The separation of digital images and clinical data guaranteed security of privacy. The involved pathologists were asked to first diagnose the case digitally and to inspect the original slides and conclusion after completion of the questionnaire only.

Digital diagnostics

The three laboratories were free in the assignment of individual cases to pathologists, however, the pathologists had to have some basic experience with digital pathology. In total, 16 pathologists were involved in the study (3 UMCG, 10 Isala and 3 LABPON). The study cases were digitally assessed using the IMS viewer (Philips). Since only cases from the other two mutual laboratories were scored, there were 180 digital assessments of the 90 cases. All pathologists were asked to score the cases blinded to the original microscopic diagnosis, but they were privy to the original clinical and macroscopic information, as well the original pathology diagnosis. They had to formulate a diagnosis, but no microscopic evaluation was requested. In addition, participants were asked to score the delay in digital case retrieval (latency) after login at the IMS server as 1 (noticeable latency), 2 (some latency) or 3 (no noticeable latency). Similarly, image build up (tiling) latency was scored in a similar way (score 1-3), and lastly, quality of focus of the images was scored from 1 (whole slide out of focus), 2 (several parts out of focus), 3 (minor parts out of focus) to 4 (everything optimally focussed).

Data analysis

In line with previous studies (7-11), the original microscopic and WSI based diagnoses were compared by at least two independent pathologists to judge if the correlation between two diagnoses were concordant (complete agreement between the first original signed out diagnosis and the WSI based diagnosis), slightly discrepant (mild differences between the two diagnostic modalities without clinical or prognostic implications), or discrepant (differences with clinical and/or prognostic implications for the patient). If possible, discordances were also classified for their cause (interpretative or more technical reasons).

RESULTS

In total 90 cases were evaluated (Table 1) resulting in 180 digital evaluations (each laboratory evaluated only the cases provided by the other two laboratories). The 90 cases encompassed 780 glass slides, 1-44 slides per cases, with a median of 6 glass slides per case. From these 780 glass slides 776 were actually scanned and available for digital analysis. The four digital slides that were missing were one iron staining, one cytological smear and two immunohistochemistry slides. This omission did not result in any diagnostic problem, so all 90 cases remained included in the series. In one slide a peripheral piece of tissue that was only partially covered by the tape was not scanned.

The pathologists scored the speed of case retrieval and of image building and focus quality. The mean values for these scores were 2.37 (scale up to 3; 1 = considerable latency to 3= no latency), 2.39 (idem) and 3.06 (scale up to 4, from score 1 = whole slide out of focus to score 4 = everything in focus), respectively (Figure 1). Focusing problems of any score were noted in 41 of digital 776 slides (5.3%). Five slides were (almost) completely out of focus (0.6%), and 9 slides were partially out of focus (1.2%). In a few cases, the focussing problems were due to ink-marks on top of the original glass slides that (on purpose) had not been removed before scanning. In one prostate biopsy with a very small focus of adenocarcinoma readily detectable in the original double staining for basal keratins and p63, one pathologist was not able to make a certain digital diagnosis of adenocarcinoma, just because of this annotation on the glass slide and the resulting blurring (Figure 2).

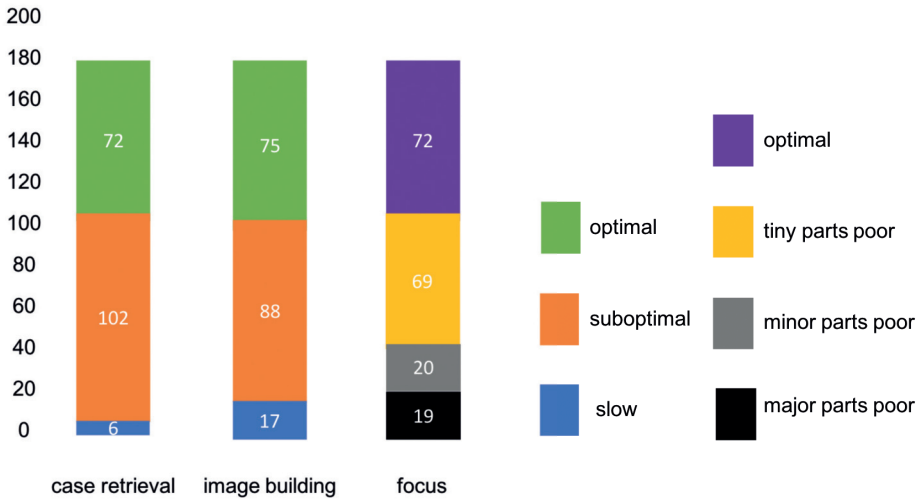


Figure 1. Speed of case retrieval and image building and quality of image focus**.**

Using the same image management system and viewer in their own laboratory, each pathologist scored the speed of digital case retrieval and image building in a 3-tiered system. The quality of focussing was scored in a 4-tiered system. Since each case was scored by two pathologists at different locations, 180 assessments were available. The number of assessments with a specific score are indicated within the coloured bars. The numbers of individual slides with focussing problems are given in the text.

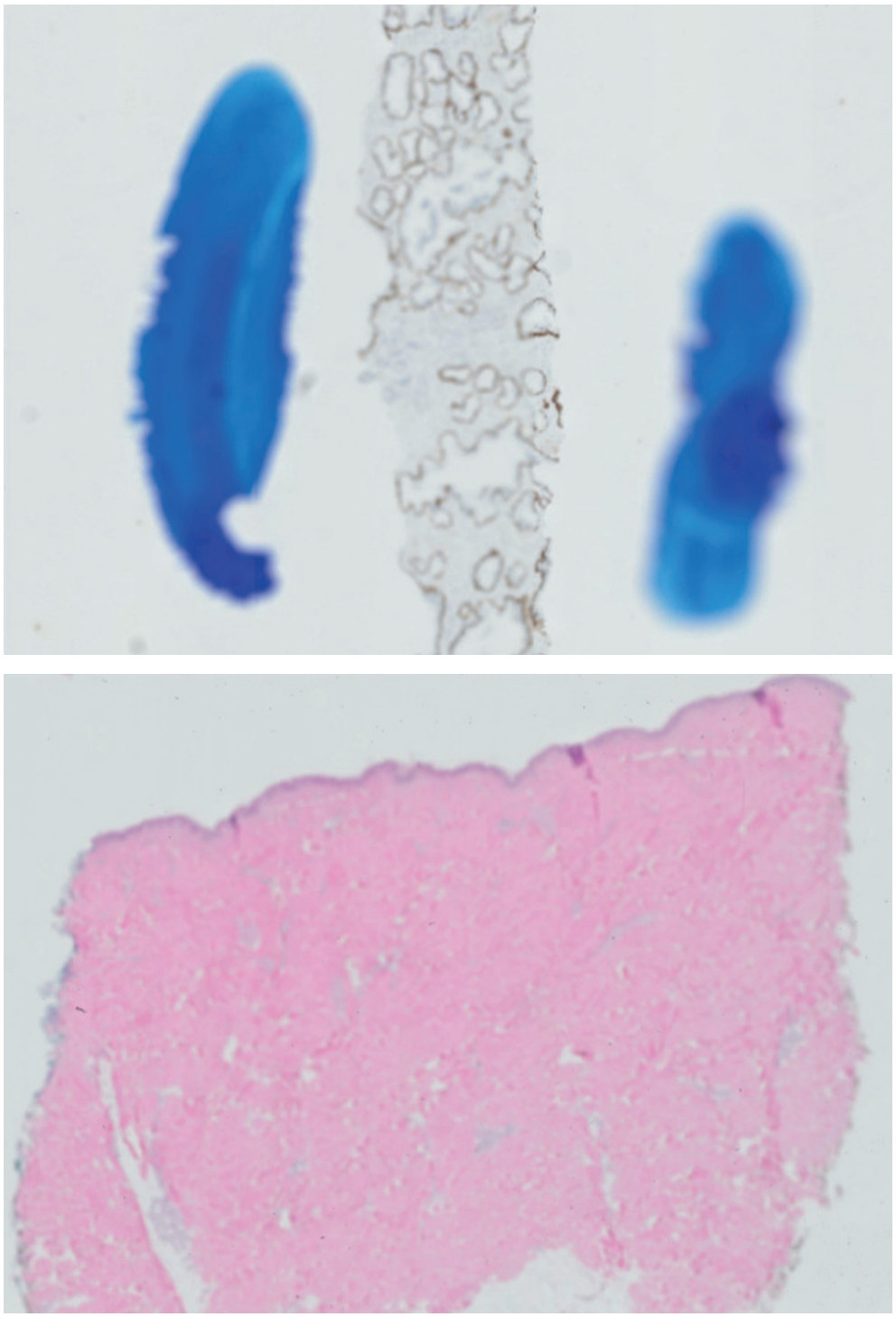


Figure 2. Slide focusing problems

Examples of a digital whole slide image with focusing problems. Top: prostate biopsy with, likely due to the blue ink marks on top of the coverslip, a poorly focussed region of focal adenocarcinoma that did preclude a correct diagnosis of for one pathologist. Bottom: poorly focused section of a skin biopsy (other serial sections of the same case were in focus).

In 80 / 90 cases (89%, 180 evaluations) full concordance was obtained between all three diagnoses (one original and two digital diagnoses per case). There were two major (2.2% of cases) and 8 minor discordances (8.8% of cases; Table 2). Further analysis showed that most discordances were likely due to differences in interpretation / experience / awareness of the pathologists (8 cases). In one skin biopsy with serial sections on 3 glass slides, a small basocellular carcinoma (BCC) was overlooked by one pathologist. One skin biopsy contained a small BCC that was surrounded by a monotonous lymphocytic infiltrate. This infiltrate had not been not immunophenotyped by the contributing laboratory since the patient was already known with B cell chronic lymphocytic leukaemia (B-CLL). One pathologist did not interpret this infiltrate as abnormal in the digitalized slides, and thus diagnosed only the BCC. In only 2 cases technical issues played a role in the discordance. These included the already mentioned prostate biopsy with the small focus of adenocarcinoma and a bone marrow trephine biopsy with signs of hyperparathyroidism and pure red cell aplasia, in which the large inclusions typical for Parvo-B19 viral infection in few (pro)erythroblasts were not identified in the digitalized H&E stained slides but were only appreciated in the immunohistochemical staining. Probably this was due to the low contrast in the digitalized H&E stained slides.

DISCUSSION

The aim of this study was to test the feasibility of a digital teleconsultation network for remote consultation in pathology using WSI. Three laboratories participated with in total 90 scanned cases. Each laboratory was asked to review the cases from the two other labs and to score relevant technical issues. The number of cases was relatively small compared to other studies, but the case mix was relatively complex, including renal, bone marrow and lymph node biopsies with malignant lymphomas, as well as a few sarcoma specimens, allowing evaluation of the network for consultation purposes. Importantly, all 16 pathologists were familiar with digital pathology but, with the exception of a few, were not daily practising it, precluding any positive prejudice to digital pathology.

The digital network indeed appeared to be suitable for consultation purposes with only a few technical issues limiting its use. In particular, the speed of case retrieval and building images were not optimal, nevertheless, not perceived as a major nuisance by the 16 involved pathologists (Figure 1). Some slides showed parts that were out of focus as well, however, focus quality was generally felt to be sufficient. In only one case with a very small focus of prostatic adenocarcinoma out-of-focus problems hindered the diagnostic process.

N	tissue type	microscopic diagnosis	digital diagnosis 1	digital diagnosis 2	preferred diagnosis	type discrepancy	cause*
1	skin resection	residual melanoma	residual in situ melanoma	no residual melanoma	microscope	major	P
2	prostate biopsy	small focus adenocarcinoma	small focus adenocarcinoma	no diagnosis	microscope	major	T
3	skin biopsy	BCC	trichofolliculoma	BCC	microscope	minor	P
4	skin biopsy	BCC & B-CLL	B-CLL	BCC & B-CLL	microscope	minor	P
5	skin biopsy	BCC & actinic keratosis	actinic keratosis	BCC & actinic keratosis	microscope	minor	P
6	lymph node	breast gland inclusion	reactive with small breast gland inclusion	reactive lymph node	microscope	minor	P
7	bone marrow	osteitis fibrosa cystica & pure red cell Bovenkant formulieraplasia based on Parvo virus infection	osteitis fibrosa cystica & pure red cell Bovenkant formulieraplasia (Parvo virus in IHC)	cyst & pure red cell Bovenkant formulieraplasia (Parvo virus in IHC)	microscope	minor	P &T?
8	liver biopsy	bile duct adenoma	bile duct hamartoma & haemangioma	bile duct cyst	microscope	minor	P
9	resection	chondrosarcoma grade 1	chondrosarcoma grade 1	enchondroma or chondrosarcoma grade 1	microscope	minor	P
10	gastric biopsy	fundic gland polyp with low grade dysplasia	fundic gland polyp	fundic gland polyp	digital	minor	P

*P: pathologist (awareness, interpretation); T: technical, image quality (focus, contrast)

Table 2. Discrepancies between digital and microscopic diagnoses.

	N (%)
Slides fully scanned in focus	735 (94.7%)
Slides with minor focusing problems	27 (3.4%)
Slides with more extensive focusing problems	14 (1.8%)
Total	776 (100%)

Table 3. Breakdown of specimens with very focal to more extensive focusing problems.

Some restraints of digital pathology for consultation and review are obvious, such as the impossibility to perform additional studies like molecular analysis, but also the use of birefringence for the detection of amyloid in Congo red stained slides. Other limitations are the detection of small microorganisms like Helicobacter Pylori in H&E slides. However, in general such limitations can be easily avoided, for instance by the addition of other stains like immunohistochemistry for Helicobacter Pylori.

One major drawback of teleconsultation may be the problematic digital transmission of patient data including the original report to the consultant pathologist. At the time of the study in 2013 and 2014, it was technically impossible to distribute clinical and relevant macroscopic data in a safe way. As a consequence, these data had to be send by post, which is the accepted way but interestingly the obvious lack of safety of regular mail is not a matter of debate. However, these safety problems of digital communication can be solved by simultaneous transmission along secured routes, e.g. via virtual private network connections or existing encrypted digital networks between individual hospitals already used in cardiology or radiology. Moreover, nationwide pathology networks such as the PALGA system in The Netherlands can be used for this purpose (www.palga.nl).

The re-diagnoses were completely concordant with the original diagnosis in 89% of the cases. When eight minor discrepancies were not taken into account the concordance rate rose to 88/90, i.e. 98%. Moreover, reanalysis of the discordances showed that only two (minor and major) discrepancies could be attributed to the use of digital pathology (2%), while the other eight discrepancies were most likely due to differences in awareness and interpretation by the individual pathologists. This high overall concordance rate is within the range of previously observed inter- and intra-observer variability in digital and microscopic pathology in general and is in line with previous single institute studies by our group [7-10] and others [11 and 15].
Although there have been many studies validating digital diagnostics, there are only a few studies specifically validating remote diagnostics. Furness et al. evaluated the adequacy of WSI as a medium for internet-based telepathology in the context of The National Renal Pathology External Quality Assurance scheme in the UK [12]. Their results showed no significant difference between the diagnostic accuracy of the pathology reports derived from WSI and conventional microscopy. In one study, WSI data were put on a hard disk and sent to an outside laboratory for referral [13]. In another study glass slides were scanned prospectively upon receipt by the consultant [14]. In other studies, cases were re-analysed digitally by expert pathologists, possibly resulting in a relatively low rate of discordance related to an incomplete wash out for these consultation cases [16]. We have not found any further WSI diagnostics validation studies focussing on remote consultation. Together, these results indicate that consultation using WSI in an inter-laboratory network is feasible and sufficiently reliable.

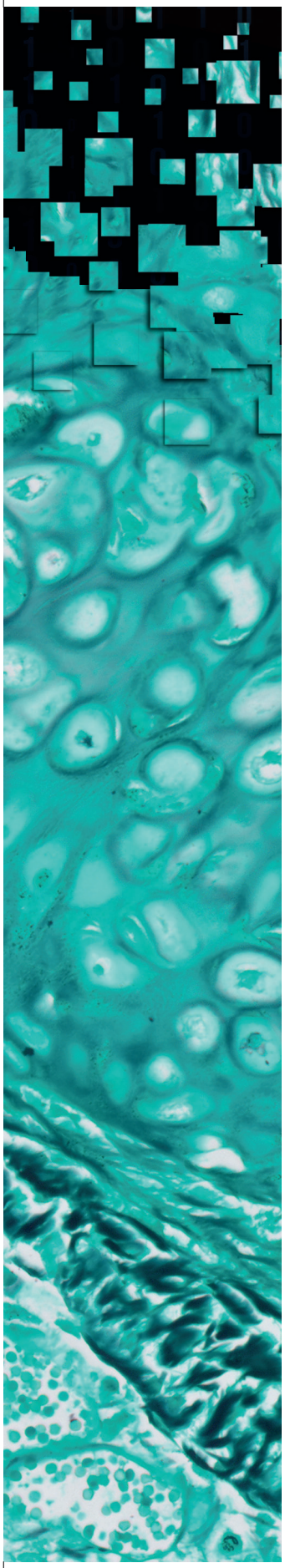
In several cases of our series the quality of images was suboptimal, in some of these cases a better-quality image was obtained after re-scanning (data not shown). In one case this led to the impossibility to appropriately diagnose a small focus of prostatic adenocarcinoma. This focussing problem was likely caused by annotations in ink on the glass slide to indicate the small spot of cancer. Annotating slides with ink is felt as very helpful to immediately attract the attention of the consultant to certain important details. Of course, this practice will be abandoned in a situation where also the primary diagnosis is made using digital pathology, since the annotations will be made digitally instead of physically. Importantly, other specimens with very focal to more extensive focussing problems (4.2% of all slides) were encountered but this did not preclude a correct diagnosis (Table 2). This relative high percentage illustrates, nevertheless, that pathologists should be critical about the focus quality of the images. Since focus problems can easily be recognized, a proper check of the focus quality of all images to be uploaded, and rescanning of slides if necessary, should be incorporated for the time being. This can be done by a technician, but technical improvements as well as software algorithms automatically checking focus quality will also likely find a place in daily practice. To date, some scanners already have these options. Rescanning slides will in practice lead to some delay but will still be much faster than sending slides by mail.

Incorporation of digital consultation in routine practice requires fast handling of images to prevent efficiency losses and match routine microscopic assessments. To evaluate the proposed system in respect to loading, zooming and focussing speed, we asked the participants to score latencies which were critical factors in handling WSI. The scores of 2.37 and 2.39 were not optimal, obviously leaving room for improvement. However, none of the 16 pathologists experienced this as a major drawback. Slow loading speed can be amended by optimizing the network and image server performance, streaming properties of the viewer, processor speed and graphics performance of the computer. The same holds for zooming latency. The rate limiting factors here are slow external and internal networks, a slow server and/ or computer.

In conclusion, this pilot study demonstrates the suitability of a teleconsultation network for fast remote digital consultation. These encouraging results motivate further regional or nationwide networks of digital pathology as presently initiated. In the Netherlands an initiative, project PIE (Pathology Image Exchange), started three years ago to realize a national platform for exchange of WSI between Dutch pathology labs for consultation, revision and pathology panels. This project is in the meanwhile operational and allows to process cases for digital revision and consultation with all the relevant patient data available in a secure environment.

REFERENCES

1. Al Janabi S, Huisman A, Van Diest PJ. Digital pathology: current status and future perspectives. Histopathol 2012; 61:1-9.
2. Cheng CL, Tan PH. Digital pathology in the diagnostic setting: beyond technology into best practice and service management. J Clin Pathol 2017; 70:454-457.
3. Pantanowitz L, Sinard JH, Henricks WH, et al; College of American Pathologists Pathology and Laboratory Quality Center. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Arch Pathol Lab Med 2013; 137:1710-1722.
4. Williams S1, Henricks WH, Becich MJ, Toscano M, Carter AB; Telepathology for patient care: what am I getting myself into? Adv Anat Pathol 2010; 17:130-149.
5. Chen J, Jiao Y, Lu C, Zhou J, Zhang Z, Zhou C; A nationwide telepathology consultation and quality control program in China: implementation and result analysis. Diagn Pathol. 2014; 10.1186:1746-1596-9.
6. Zhao C, Wu T, Ding X, et al. International telepathology consultation: Three years of experience between the University of Pittsburgh Medical Center and KingMed Diagnostics in China.; J Pathol Inform. 2015; 10.4103: 2153-3539.
7. Al Janabi S, Huisman A, Vink A, et al. Whole slide images for primary diagnostics in dermatopathology: a feasibility study. J Clin Pathol 2012; 65:152-158.
8. Al Janabi S, Huisman A, Vink A, et al. Whole slide images for primary diagnostics of gastrointestinal tract pathology: a feasibility study. Hum Pathol 2012; 43:702-707.
9. Al Janabi S, Huisman A, Nap M, Clarijs R, van Diest PJ. Whole slide images as a platform for initial diagnostics in histopathology in a medium sized routine laboratory. J Clin Pathol 2012; 65:1107 1111.
10. Al Janabi S, Huisman A, Willems SM, Van Diest PJ. Digital slide images for primary diagnostics in breast pathology: a feasibility study. Hum Path 2012; 43:2318 2325.
11. Goacher E, Randell R, Williams B, Treanor D. The Diagnostic Concordance of Whole Slide Imaging and Light Microscopy: A Systematic Review. Arch Pathol Lab Med. 2017; 141:151-161.
12. Furness P. A randomized controlled trial of the diagnostic accuracy of internet-based telepathology compared with conventional microscopy. Histopathology 2007; 50:266-273.
13. Wilbur DC, Madi K, Colvin RB, et al; Whole-slide imaging digital pathology as a platform for teleconsultation: a pilot study using paired subspecialist correlations. Arch Pathol Lab Med 2009; 133:1949-1953.
14. Jones NC, Nazarian RM, Duncan LM, et al; Interinstitutional whole slide imaging teleconsultation service development: assessment using internal training and clinical consultation cases. Arch Pathol Lab Med 2015; 139:627-35.
15. BJ Williams et al; A systematic analysis of discordant diagnoses in digital pathology compared with light microscopy. Arch Pathol Lab Med 2017; 141:1712-1718.
16. Bauer TW, Slaw RJ; Validating whole-slide imaging for consultation diagnoses in surgical pathology. Arch Pathol Lab Med 2014; 138:1459-1465.



CHAPTER 4

Evaluating the benefits of digital pathology implementation: time savings in laboratory logistics

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ABSTRACT

Background: The benefits of digital pathology for workflow improvement and thereby cost savings in pathology, at least partly outweighing investment costs, are being increasingly recognised. Successful implementations in a variety of scenarios have started to demonstrate the cost benefits of digital pathology for both research and routine diagnosis, contributing to a sound business case encouraging further adoption. To further support new adopters, there is still a need for detailed assessment of the impact that this technology has on the relevant pathology workflows, with an emphasis on time-saving.

Aims: To assess the impact of digital pathology adoption on logistic laboratory tasks (i.e. not including pathologists' time for diagnosis-making) in the Laboratorium Pathologie Oost Nederland, a large regional pathology laboratory in The Netherlands.

Methods and results: To quantify the benefits of digitisation, we analysed the differences between the traditional analogue and new digital workflows, carried out detailed measurements of all relevant steps in key analogue and digital processes, and compared the time spent. We modelled and assessed the logistic savings in five workflows: (i) routine diagnosis; (ii) multidisciplinary meeting; (iii) external revision requests; (iv) extra stainings; and (v) external consultation. On average, >19 working hours were saved on a typical day by working digitally, with the highest savings in routine diagnosis and multidisciplinary meeting workflows.

Conclusions: By working digitally, a significant amount of time could be saved in a large regional pathology laboratory with a typical case mix. We also present the data in each workflow per task and concrete logistic steps to allow extrapolation to the context and case mix of other laboratories.

INTRODUCTION

Working with digital whole slide images instead of the traditional microscope, usually denoted digital pathology, has many advantages and has been widely accepted for research and education.[1] The implementation of a fully digital workflow for diagnostic purposes poses several important challenges to its implementation in diagnostic surgical pathology: workflow integration, technological infrastructure, pathologist acceptance, and standardisation. Also, investment and implementation costs are significant, necessitating the creation of a business case for which there is, besides an increase in the quality of diagnoses, a return on investment. A diagnostic digital pathology infrastructure may facilitate substantial workflow improvements that may lead to higher efficiency, better utilisation of resources, higher throughput, and a lower turnaround time of cases, and also a streamlined collaboration both within a single pathology laboratory and across organisational boundaries, and thereby lower costs.[2,3]

Only a few previous studies have addressed whether a digital workflow is or could be more efficient. Ho *et al.*[4] focused on identifying the needs of the pathologists and on designing the digital workflows to best address these needs. A total of six pathologists were interviewed and observed in a large academic medical centre. The study found the analogue workflow to be labour-intensive and to lack scalability. Several workflows that would benefit from the introduction of digital pathology were identified: case management, case examination and review, and final case reporting. In the study of Vodovnik,[5] digital pathology required a shorter diagnosis time than traditional microscopy in 13 of the 20 diagnostic sessions after completion of an initial learning phase, optimisation of the diagnostic setting with a fully integrated laboratory management system, the installation of double displays, and the creation of a stable network. This was related to the absence of physical slide-handling and the consolidation of multiple tasks in digital reporting systems. This confirmed the potential of digital pathology to yield savings in both diagnostic and non-diagnostic tasks.

Ho *et al.*[6] described an economic impact model for a very large pathology department that receives 219 000 cases annually and employs a network of pathologists located at both academic and community-based hospitals across a large geographical area. The potential operational cost savings for 5 years following the implementation of a digital pathology solution were estimated to be approximately $18 million. The main contributory factors were gains in pathologist time resulting from higher productivity and better workload distribution ($12.4 million), and reduced costs of incorrect treatment. The overtreatment and undertreatment costs in oncology were estimated to be $5.4 million. Workflow improvement benefits were also identified, including a refinement of the current 'centre of excellence' model and the ability to train all pathologists in the network in subspecialties based on the ability to distribute cases across the network. Furthermore, with the digital solution, pathologists at smaller hospitals could receive sufficient cases to train in subspecialties.

To further strengthen the literature and contribute to the evidence required to build a vital business case for digital pathology, we here report on the time savings following the adoption

of digital pathology at the Laboratorium Pathologie Oost Nederland (LabPON), a large regional pathology laboratory in The Netherlands that was among the first laboratories to create a fully digital diagnostic workflow.[7]

METHODS

With the adoption of a digital pathology solution, several steps in the workflow change and some of the human tasks are replaced by automatic digital transmission. Digitisation also enables the integration of new applications in the workflow that were not available before, such as automatic case distribution, automatic image analysis, and remote diagnosis. The implementation of digital pathology diagnostics therefore forced us to re-evaluate our pathology diagnosis processes to arrive at an optimal digital workflow. In the process of going digital, we faced several technical challenges in the laboratory with regard to slide coverslipping, drying, and labelling, adapted workspaces and hardware of the pathologists, and installed storage, as detailed previously.[7] The network went down only once at the start of the project, because of server collapse, but has performed surprisingly well ever since.

To accurately and systematically assess the differences between the digital and analogue laboratory processes with respect to logistic steps, we modelled the key workflows in the pathology laboratory that are affected by the transition to digital. The selected workflows are: (i) routine diagnosis (Figure 1); (ii) multidisciplinary meetings; (iii) external revisions (Figure 2); (iv) extra stainings (Figure 2); and (v) consultations (Figure 2). The time needed for pathologists to make diagnoses was outside the scope of the current study. Next, we identified task overlaps across these workflows to avoid accounting for the same task multiple times, as some tasks occur in multiple workflows.

Once we had identified the relevant tasks affected by digitisation, we measured their durations in the conventional (analogue) pathology setting during several sessions on days with a typical workload for LabPON. Similarly, we measured the duration of the corresponding tasks (if still present) or amended tasks in the digital workflows. The measurements of activities of the secretary, dispatchers and archivists were made over a period of 12 days with a typical workload, resulting in >10 full days of measurements. For these three groups, two people were monitored in half-day periods to record all relevant activities.

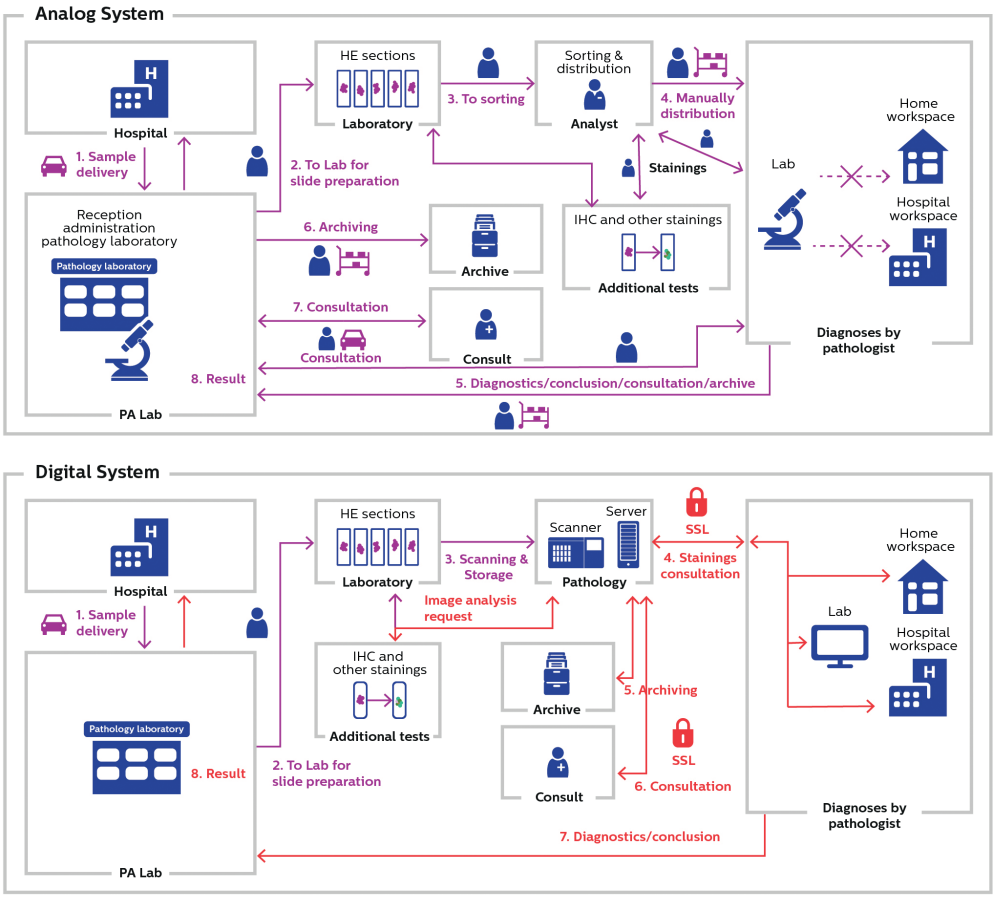


Figure 1. Analogue and digital workflows in the Laboratorium Pathologie Oost Nederland. In the digital workflow, several logistic steps are eliminated.

Tasks that were eliminated in the digital workflow were set to a duration of 0 s, and, for the remaining tasks, the analogue measurements were used. For amended tasks in the digital workflow (e.g. transport from administration to pathologists), the accurate time measurements were assigned. These measurements were facilitated by the fact that, whereas LabPON is almost fully digital with respect to diagnosis-making by pathologists, it still maintains the analogue workflows. The workflows were modelled with the Business Process Model and Notation (BPMN2.0) standard, which has been used to model anatomical pathology processes before.[8]

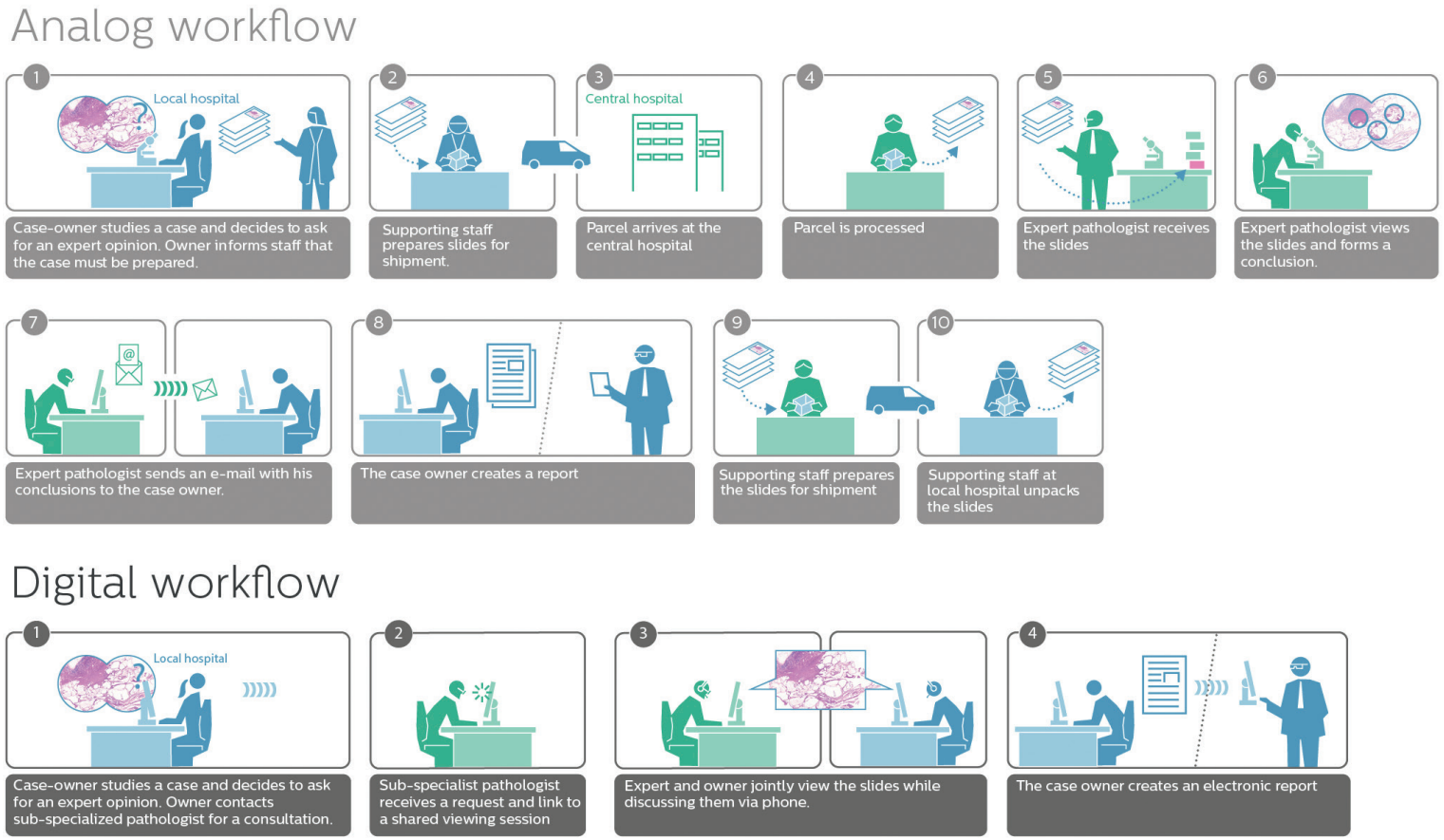


Figure 2. Analogue and digital workflows in the Laboratorium Pathologie Oost Nederland for (external) consultation. In the digital workflow, the glass slides do not need to be sent to the external site, but the case can be reviewed by the expert by accessing a link to a shared viewing session. This eliminates the risk that glass slides will be damaged during transportation, reduces the administrative burden, and improves turnaround times.

RESULTS

On a typical working day, 220 new cases are produced at LabPON. In the analogue workflow, the urgent cases (10%) go directly to the pathologists after being processed, whereas the rest are dispatched in batches. The dispatching takes place eight times per day and takes 10–15 min. Figures 3–7 and the corresponding Tables 1–5 depict the five analogue workflows annotated with the total durations per day of the relevant tasks.

In Figure 3 and Table 1, measurements of the logistics task durations in the routine diagnosis workflow are compared with those in the digital workflow. The 'prepare case' task in the digital workflow (corresponding to step A in the analogue workflow) includes putting the slides in the scanner, removing them from the scanner and putting them on the slide transportation trolley, and finally bringing the trolley to the archive for the daily average of about 220 cases. This amounts to approximately 32 min for the preparation of the 220 cases and 2–4 min for bringing them to the archive (one or two times per day), adding up to around 34–36 min/day. Therefore, from step A in the analogue workflow, we may save around 484 min/day. Steps B and C of the analogue workflow could be entirely removed and replaced with digital transmission and handling, leading to savings of about 175 min/day. Step D remains in the digital workflow (putting the physical slides in the archive). Therefore, from the routine diagnosis workflow, we can save 659 min/day by going digital, which corresponds to around 3 min/case.

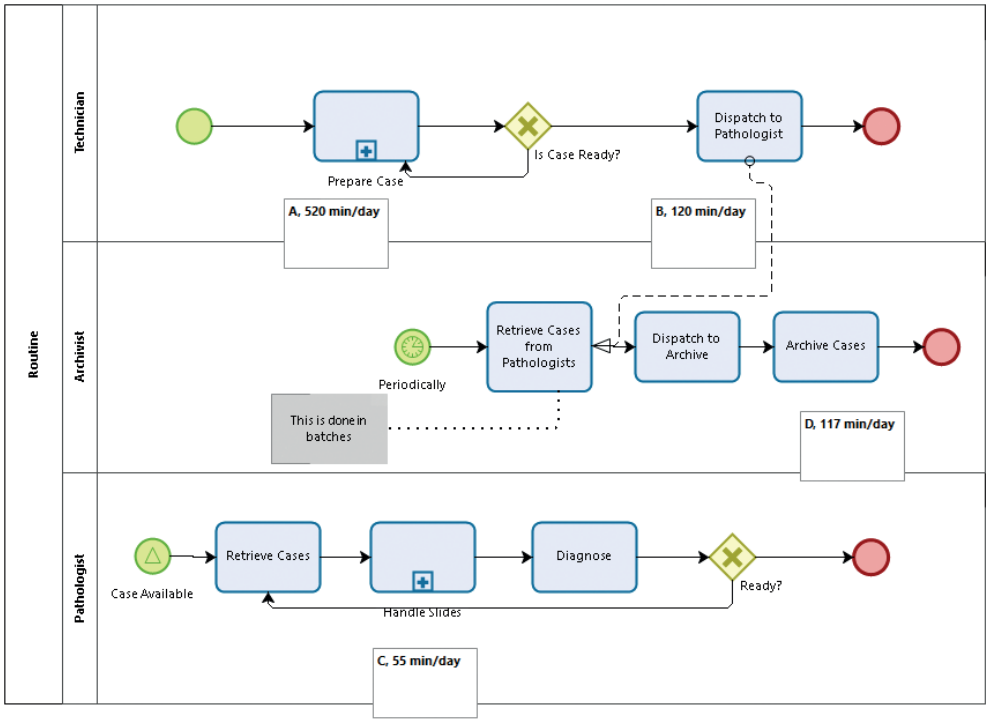


Figure 3. Workflow diagram. Analogue workflow of routine diagnosis with the measured durations of key tasks. See Table 1 for detailed time measurements in both the analogue and digital situations.

Task	cases/day	Activity	By	Time Per Day analog	Time per day digital
A	220	Prepare (find form - retrieve case - scan barcode - put to folder)	Tech	520	36
B	198	Transfer cases to pathologist with the slide transportation trolley (in batches)	Arch/Tech	120	0
C	220	Case handling by pathologist. Retrieve from case - sort cases - inspect - open - scan barcode (slide handling) - close case	Path	55	0
D	220	Archive (archivist puts slides in folders and places them in the archive)	Arch	117	117
			Total	**812**	**153**

Table 1. Analog workflow of routine diagnosis with the measured duration of key tasks. Detailed time measurements both in the analog and digital situation. Please see figure 3 for workflow diagram.

In Figure 4 and Table 2, we focus on the multidisciplinary meetings preparation workflow. Every day, three or four multidisciplinary meetings are prepared, and each meeting concerns, on average, 17 cases (with a range from five to 30 cases). In the multidisciplinary meeting, workflow digitisation completely saves steps M, G, and K, amounting to 139 min/day.

The time needed by the administration to prepare the case list for multidisciplinary meetings in a digital setting was estimated to be around 7 min per meeting (5 min for small meetings to 10 min for large meetings). In total, this will amount to around 21–28 min/day, on average about 25 min. Therefore, in step F we save about 188 min/day as compared with the analogue workflow, leading to savings of about 327 min/day.

However, some cases are not yet in the archive when the multidisciplinary meeting is planned. In the analogue setting, these cases need to be found in the pathologist room. On average, this happens in about 10 cases per day and takes 4 min/case, amounting to 40 min. Therefore, the savings in this workflow can even amount to 367 min/day.

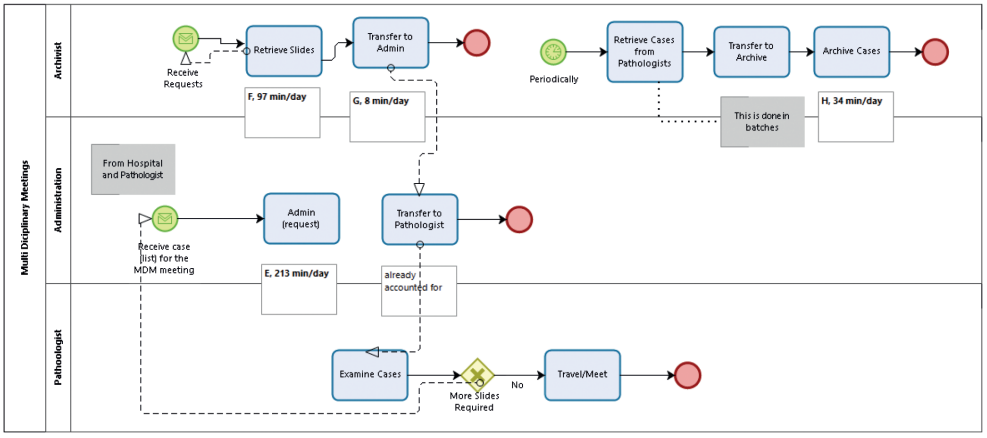


Figure 4. Workflow diagram. Analogue workflow of multidisciplinary meetings with the measured durations of key tasks. See Table 2 for detailed time measurements in both the analogue and digital situations.

Task	Cases/day	Activity	By	Time per day analog	Time per day digital
E	65	Admin (print / identify / mark) - request slides to archivist - prepare package after slides are received from archivist	Adm	213	25
F	65	Collect from archive and anywhere else	Arch	97	0
G	65	Direct transfer to admin / secretary	Arch	8	0
H	65	Archive (put case back into archive)	Arch	34	0
			Total	**352**	**25**

Table 2. Analog workflow of multi-disciplinary meeting with the measured duration of key tasks. Detailed time measurements both in the analog and digital situation. Please see figure 4 for workflow diagram.

In Figure 5 and Table 3, we focus on the external requests workflow, covering incoming requests for revisions when a patient is referred to another hospital or when material is requested for panel or research purposes. On a typical working day, LabPON receives six external requests to review cases or material. Each case is screened by a pathologist (can be the same who diagnosed it). Using digital pathology, we completely save steps H, Q, and R, amounting to 24 min/day. Step P is reduced to 10 min, leading to a 31-min saving, giving a total saving of 55 min/day. This workflow requires the external pathologist to assess the case digitally as well.

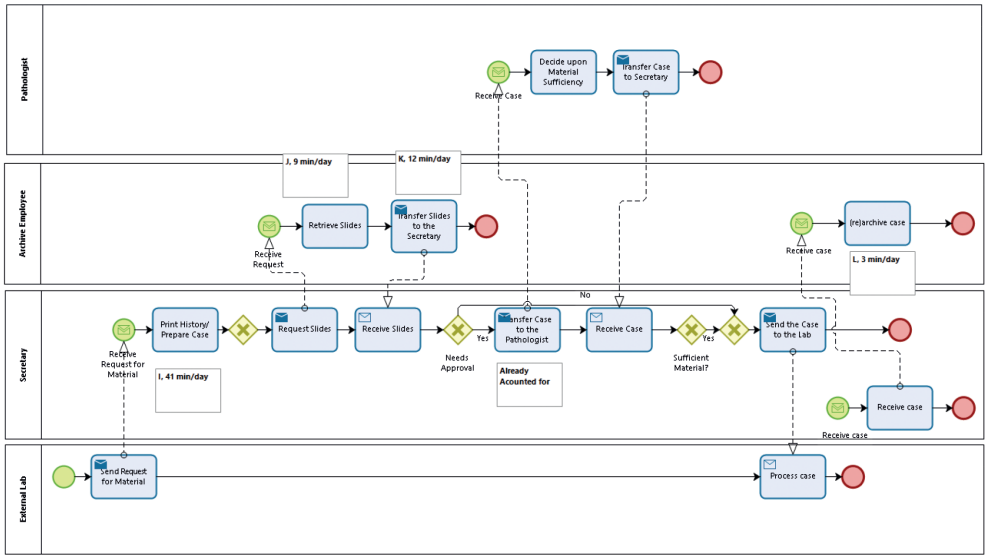


Figure 5. Workflow diagram. Analogue workflow of external revision requests with the measured durations of key tasks. See Table 3 for detailed time measurements in both the analogue and digital situations.

Task	Cases/day	Activity	By	Time per day analog	Time per day digital
I	6	Admin (check papers) - order slides and materials - prepare package when received from archive	Adm	41	10
J	6	Receive request from secretary and retrieve from archive	Arch	9	0
K	6	Direct transfer to admin / secretary	Arch	12	0
L	6	Put case back into archive when received back	Arch	3	0
			Total	**65**	**10**

Table 3. Analog workflow of external revision requests with the measured duration of key tasks. Detailed time measurements both in the analog and digital situation. Please see figure 5 for workflow diagram.

Figure 6 and Table 4 show the measurements for the extra stainings workflow. On a typical day, the pathologists at LabPON order extra stainings for 35 cases (16% of the 220 cases), 10 of which are urgent (about 29%). As in the case of routine workflow, the digital preparation (e.g. digital quality control instead of quality control under the microscope) will take less time in this workflow, amounting to around 5 min for the 35 cases. In the workflow in Figure 6, step N remains and we can save 47 min/day in step I.

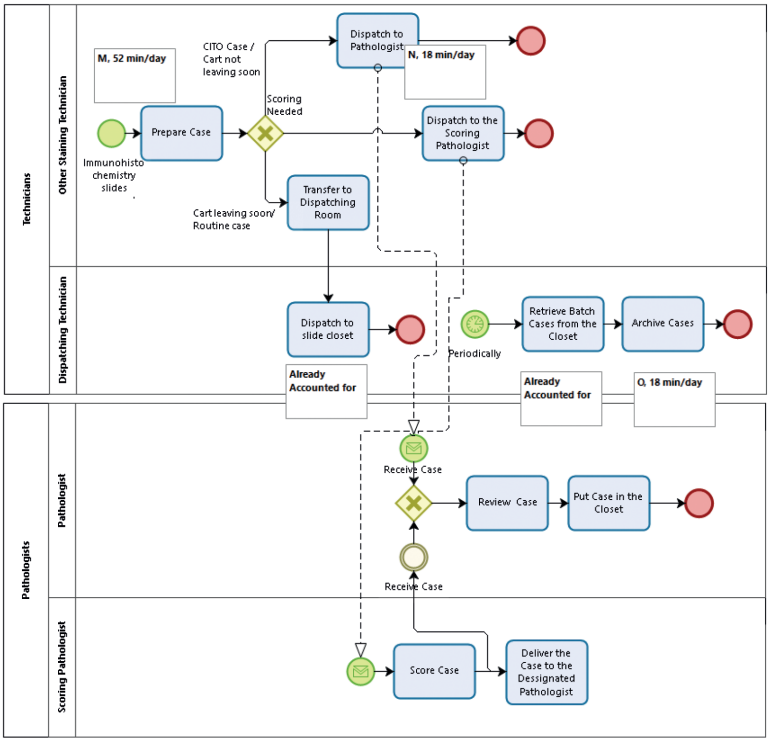


Figure 6. Workflow diagram. Analogue workflow of extra staining with the measured durations of key tasks. See Table 4 for detailed time measurements in both the analogue and digital situations.

Task	Cases/day	Activity	By	Time per day analog	Time per day digital
M	35	Prepare case (staining, processing in the lab)	Tech	52	5
N	10	Transfer directly to pathologist when urgent or to dispatching room otherwise	Tech	16	16
O	35	Archive (place slides in folder, put into archive)	Arch	18	18
			Total	**86**	**39**

Table 4. Analog workflow of extra-staining with the measured duration of key tasks. Detailed time measurements both in the analog and digital situation. Please see figure 6 for workflow diagram.

Figure 7 and Table 5 detail the relevant logistic tasks in the consultation workflow. All pathologists together at LabPON typically make six requests for consultation each day, two of which concern an external pathologist, and four of which are internal requests to a colleague at LabPON. This process assumes that the external pathologist uses a digital system as well. In the workflow in Figure 7, the transition to digital saves steps O and M, giving a total of 19 min/day. Step P remains and focuses on the initiation of the request.

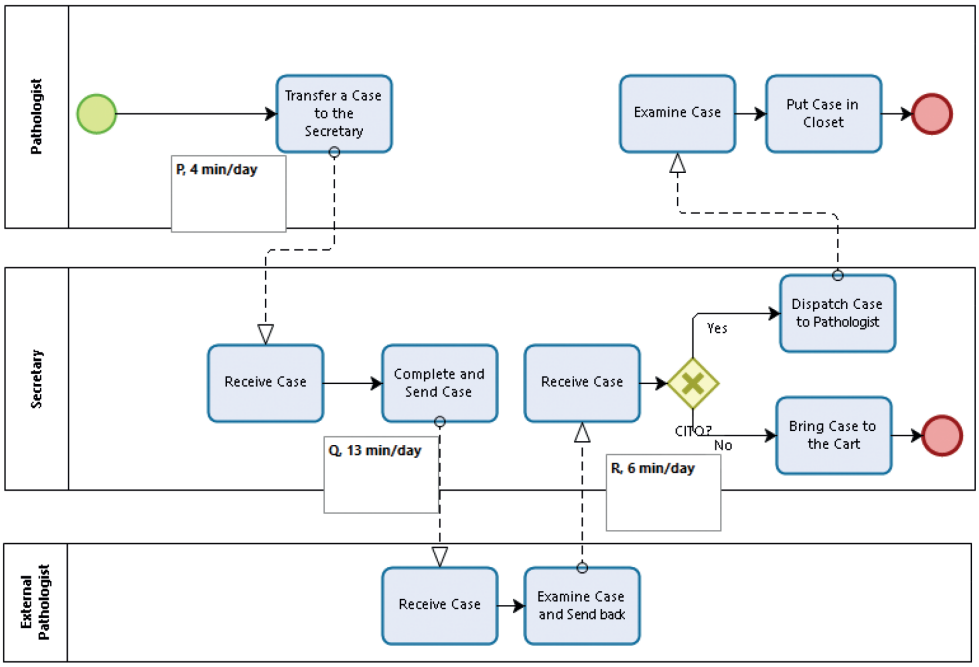


Figure 7. Workflow diagram. Analogue workflow of external consultations with the measured durations of key tasks. See Table 5 for detailed time measurements in both the analogue and digital situations.

Task	Cases/day	Activity	By	Time per day analog	Time per day digital
P	2	Write request (initiate)	Path	4	4
Q	2	Secretary print / request the consult to external and send case	Adm	13	0
R	2	Receive case back from external reviewer and dispatch into routine flow	Adm	6	0
			Total	**23**	**4**

Table 5. Analog workflow of external consultations with the measured duration of key task. Detailed time measurements both in the analog and digital situation. Please see figure 7 for workflow diagram.

Thereby, in total, digitisation saves about 1147 min/day in the five key workflows, which is >19 h for a typical day. This amounts, on average, to >5 min saved in logistics for each case diagnosed. Saving about 19 h/day translates into 2.63 fte (fulltime-equivalent) laboratory staff (36-h working week), corresponding to €120 000/year.

Laboratory staff and administration were soon very happy with the efficiency of the system as a whole. Pathologists were *a priori* less enthusiastic, as some feared that it would take them longer to complete their diagnosis, but this turned out not to be the case. After the training period, all agreed that working digitally is, on average, faster.

Clinical colleagues were, from the beginning, very positive about our switch to digital. Multidisciplinary meetings were more often illustrated with pathology images, which increased the level of discussion and mutual understanding; cases could be discussed *ad hoc* during the meeting as well. Moreover, additional questions could be answered on the spot over the telephone, as slides no longer needed to be retrieved from the archive, and full images and snapshots could be shared much more easily.

DISCUSSION

The aim of this study was to assess the impact of digital pathology adoption on logistic laboratory tasks in a large regional pathology laboratory in The Netherlands. We modelled and analysed, for the five most common workflows, the differences between the traditional analogue way and the new digital way of working, carried out detailed measurements of all relevant steps in key analogue and digital processes, and compared the time spent. On average, leaving out the time that individual pathologists needed for their evaluation of slides, in total >19 h (of which ~1 h was pathologists' time) was saved on a typical day by working digitally, with the highest savings in the routine diagnosis and multidisciplinary meeting workflows. As far as we know, this is the first study on these important logistic aspects of digital pathology.

The current study provides the data from a large regional laboratory, but the way in which we model the different steps and provide a detailed view of the different logistic activities and their ratio in the overall workflow allows us to extrapolate estimated savings to laboratories with different volumes and workflows in each category. For instance, on the basis of these numbers, a good estimation can be made of the amount of savings in a laboratory that has a different ratio of routine versus external consultation workflow cases.

The described time savings benefited the department. Basically, we now perform more work with the same number of staff. In particular, since 2015, when we started the project, there has been an increasing demand on multidisciplinary conferences for patient care in the multiple hospitals that LabPON is serving. At that time, we were slightly understaffed, so time savings were used to balance this out. When we eventually go fully digital (all pathologists 100% and including cytology), we expect to have an even greater benefit from the optimised logistics.

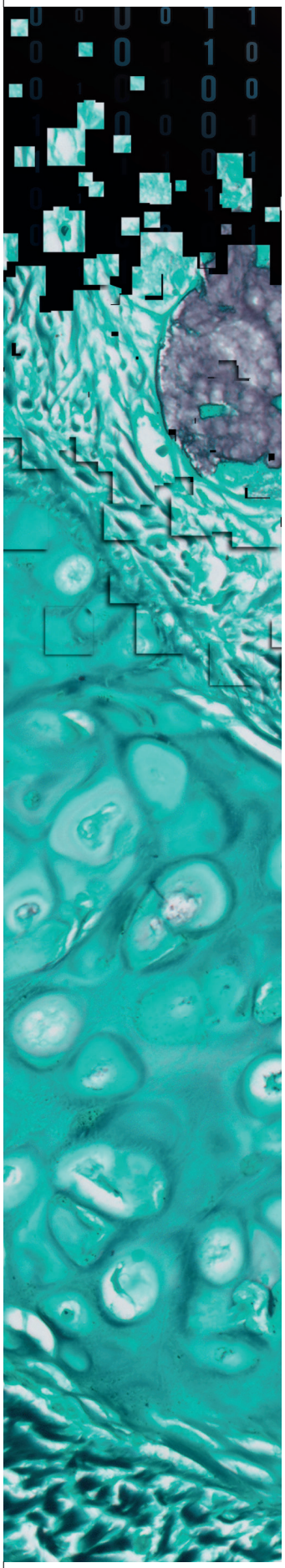
On top of the described savings, we expect additional time savings for specific, often low-volume, subspecialties such as nephropathology, for which pathologists of smaller laboratories could digitally consult experienced pathologists in larger laboratories such as LabPON and/or participate in regular digital meetings to discuss the cases. Moreover, as most countries face a shortage of pathologists and a higher demand on specific knowledge in all areas of pathology, a need to optimise the time spent on reading cases and participating in conferences (including the travelling time to hospitals), will be highly desirable in the coming years. The general perception in our department is that working digitally is faster, but a formal evaluation of whether the diagnostic process performed by pathologists is itself is more efficient when they are working digitally will be the subject of a future study.

We would like to point out that savings in logistics, and perhaps also in time spent by the pathologists, will probably not be enough to arrive at a financially neutral business case for digital diagnostic pathology, as the investments in hardware and software are significant. However, we argue that a business case should not only contain financial items, but should also be concerned with increased user satisfaction of pathology and clinical staff, greater attraction of young doctors to the residency programme, increased speed of diagnosis (and thereby lower anxiety and work dropout of patients and their relatives), and last, but not least, high patient safety and increased quality of diagnosis, saving significant costs outside the pathology laboratory, as pointed out by Ho *et al*.

In conclusion, a significant amount of time can be saved in a large regional laboratory for a typical case workload by working digitally. Our in-depth analysis of all components may help other laboratories to introduce digital pathology, and to justify the investments.

REFERENCES

1. BPMN Standard. Available from: http://www.bpmn.org
2. Jara-Lazaro AR, Thamboo TP, Teh M, Tan PH. Digital pathology: exploring its applications in diagnostic surgical pathology practice. *Pathology* 2010; 42**;** 512–518.
3. Stathonikos N, Veta M, Huisman A, van Diest PJ. Going fully digital: perspective of a Dutch academic pathology lab. *J. Pathol. Inform.* 2013 Jun 29; 4:15.
4. Ho J, Aridor O, Parwani AV. Use of contextual inquiry to understand anatomic pathology workflow: implications for digital pathology adoption. *J. Pathol. Inform.* 2012 Sep 28; 3:35.
5. Vodovnik A. Diagnostic time in digital pathology: a comparative study on 400 cases. *J. Pathol. Inform.* 2016 Jan 29; 7:4.
6. Ho J, Ahlers SM, Stratman C *et al*. Can digital pathology result in cost savings? A financial projection for digital pathology implementation at a large integrated health care organization. *J. Pathol. Inform.* 2014 Aug 28; 5:33.
7. White paper. Available from: https://thepathologist.com/fileadmin/issues/App_Notes/0016-022-app-note-How_to_Go_Digital_in_Pathology.pdf
8. Rojo MG, Rolón E, Calahorra L *et al*. Implementation of the Business Process Modelling Notation (BPMN) in the modelling of anatomic pathology processes. *Diagn. Pathol.* 2008 Jul 15; 3:22



CHAPTER 5

A whole-slide imaging based workflow reduces the reading time of pathologists

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Submitted

ABSTRACT

Even though entirely digitized microscopic tissue sections (whole slide images) are increasingly being used in histopathology diagnostics, little data is still available on the effect of this technique on pathologists' reading time. This study aimed to compare the time required to perform microscopic assessment by pathologists between a conventional workflow (using an optical microscope and glass slides) and digitized whole slide images (WSI). The study was performed in the Laboratory for Pathology Eastern Netherlands (LabPON, Hengelo, The Netherlands), which has been using WSI in primary diagnostics for several years. Cases were either read in a conventional workflow, while the pathologist recorded the time required for diagnostics and reporting or were read using a fully digitized workflow. The digitized workflow was fully integrated with the laboratory information system, and reading times were extracted from the log files of the image management system. Analysis of over 3800 cases showed a time gain in favour of the digital workflow for most case categories, with the highest gain (68% time gain) for prostate biopsies (prostate care program). On average, a gain of 12.3% was found for the digital workflow, taking the case distribution into account. It was concluded that transitioning from conventional microscopy to use of WSI significantly reduces the pathologists' reading times. A fully integrated pathologist working environment is required to fully benefit from the benefits of a digital workflow.

INTRODUCTION

In the last decade, the field of pathology has witnessed the introduction of devices that can digitize entire microscope slides at a resolution yielding sufficient quality to be used for primary diagnostics. This technology, referred to as 'whole slide imaging', brings numerous advantages but at the same time poses challenges. On one hand, whole slide imaging enables a diagnostic workflow in which no physical glass slides have to be distributed among pathologists, are damaged or lost, or have to be retrieved from the archive. Instead, digital images are easily distributed, retrieved from a digital storage, and offered to colleagues (even remotely) for consultation [1]. It strongly facilitates expert consultation and revision in case of patient referral, potentially increasing both efficiency and quality of the entire diagnostic chain. On the other hand, introduction of whole slide imaging is hampered by the fear of making incorrect diagnoses and by high additional costs associated with the digitization of microscope slides [1].

Many studies focused on the first issue: can we safely replace traditional optical slide microscopy by reading of whole slide images (WSI). In 2016, the first WSI device received clearance from the US Food and Drug Administration to be marketed as an alternative means of slide reading by pathologists. This is indicative of the existence of a large body of evidence on the non-inferiority of using WSI for diagnosis compared to the use of an optical microscope [2]. While this resolves the first issue mentioned above, wide-scale introduction of WSI is still not a reality, mainly because of the associated costs. Little research has been performed on the financial consequences of introduction of WSI in primary diagnostics.

It is relatively straightforward to calculate the costs of digitizing the entire pathology workflow, although some 'hidden' costs may be harder to assess. The financial gains, on the other hand, are more diffuse and harder to establish. Two major sources of potential financial gain are workflow logistics and diagnostic efficiency of pathologists [1]. The former has been addressed by us before [3] and the present study focused on the latter: the time required by the pathologist to sign out a case. Very little data is currently available on the effect of introduction of WSI on pathologists' reading time. Preliminary data, published already in 2010, showed a potential efficiency gain of about 14% based on extrapolation of radiology data and limited observations of pathologists [4]. In a study on 510 surgical cases, WSI reading times required on average 4 seconds per case more than microscope assessment [5], but reading time decreased significantly with increased experience in WSI assessment.

The current study was performed in one of the first pathology laboratories worldwide that has adopted a fully digitized workflow, offering a unique opportunity to research the influence of this transition on the time required for diagnosis making. The log files of the digital image management system were studied and compared with data collected from pathologists using an optical microscope in the same diagnostic setting.

MATERIALS AND METHODS

Experimental set up and case inclusion

This study was performed in the Laboratory for Pathology Eastern Netherlands (LabPON, Hengelo, The Netherlands). During the time of this study, LabPON performed primary reading of cases partly using WSI and partly using conventional microscopy. The study consisted of two independent arms: digital reading of slides and conventional reading of slides. This study was performed using consecutive cases offered for diagnostics during the study period, resulting in randomly distributed cases over the two arms.

Time required for the entire microscopic case reading by the pathologist, excluding possible consultation of colleagues, was registered. This study did not involve any patient related data nor influenced the diagnostic process, obviating the requirement for ethical approval.

Conventional slide reading

A detailed breakdown of the workflow of pathologists in a routine clinical setting based on conventional light microscopy (further referred to as 'conventional' workflow) is shown in Figure 1. In brief, a pathologist receives a stack of case folders holding one or multiple glass slides for each case. Based on the reception date of the tissues and complexity of the cases, the pathologist prioritized the cases. Next, diagnostics was done as usual on a case by case basis. The red rectangle in Figure 1 shows the workflow executed for every single case. Data are first retrieved from the lab management system (Poema, Finalist, Groningen, The Netherlands), containing patient data including patient history, clinical question, and information from grossing. Next, slides were microscopically assessed after which the diagnostic report was produced.

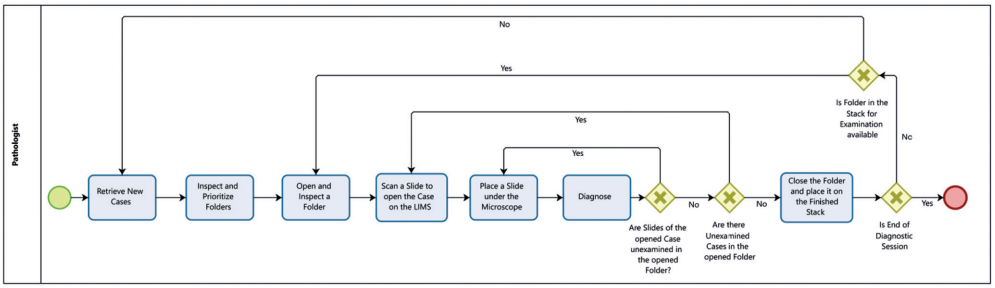


Figure 1. Overview of the diagnostic workflow using a conventional light microscope by the pathologist. The red box indicates the part of the workflow that was analysed in this study.

In this study, the time required per case was recorded using the standard barcode attached to each slide. This included the reading time for all slides of a case (including immunohistochemistry) as deemed necessary by the pathologist. The barcodes were manually scanned by participating pathologists (n = 4) prior to microscopic assessment and after finishing a reading session for a particular case. All data were digitally recorded, enabling detailed analysis of time-per-case afterwards. Also, sub-analyses for individual pathologists and case categories was performed.

Digital slide reading

Starting in 2015, LabPON gradually adopted a fully digitized workflow using WSI scanners (IntelliSite Ultra Fast Scanner, Philips, Best, The Netherlands) and the Philips IntelliSite image management system (IMS) [6]. The diagnostic workflow of the pathologist was facilitated by the IntelliSite Pathologist Suite (Figure 2). Figure 2 shows the case list window (left panel) displaying the list of assigned cases with thumbnails of the slides. The case diagnosis window (Figure 2; right panel) shows scanned slides at high resolution, enabling zooming, creation of annotations and tags. This workflow will be referred to as 'digital' workflow. A total of five pathologists participated in this arm of the study, with varying experience in primary digital reading of cases (one pathologist >2 years, one pathologist >1 year and 3 pathologists with at least 6 months experience).

The analysis of the time required per case for the digital workflow in this study was based on the IMS log files. The log files contained data on the interactions of the pathologist with the IMS as well as internal machine generated messages. As these files were not produced for this specific purpose, post-processing of the log file data was required. To be able to separate the data required for this study from the bulk of available data, specific data filters were designed for the present study.

Post-processing of ims log files

Reading of a single case may comprise multiple reading sessions when the case cannot be finished in one session. Duration of different sessions for one case were all taken into account in this study. Opening of a reading session for a case is a clearly defined event in the log files. Closing of a reading session was only explicitly coded in the log file if the pathologist changed the state of the case to 'finished' or 'review'. However, finishing a reading session that did not end with one of these events is not specifically coded in the log files. Therefore, the last action of a pathologist before opening the next case was considered to indicate the end of a reading session for a case.

In approximately 35% of sessions no image was opened. In 26% of these, the pathologist only changed the state of the case to 'review' or 'finished'. In the remaining 74% of sessions the pathologist did not use the case diagnosis window, indicating that no digital inspection was performed. These sessions were therefore disregarded in the present study. Also, sessions in which cases with state 'finished' were re-opened were not taken into account.

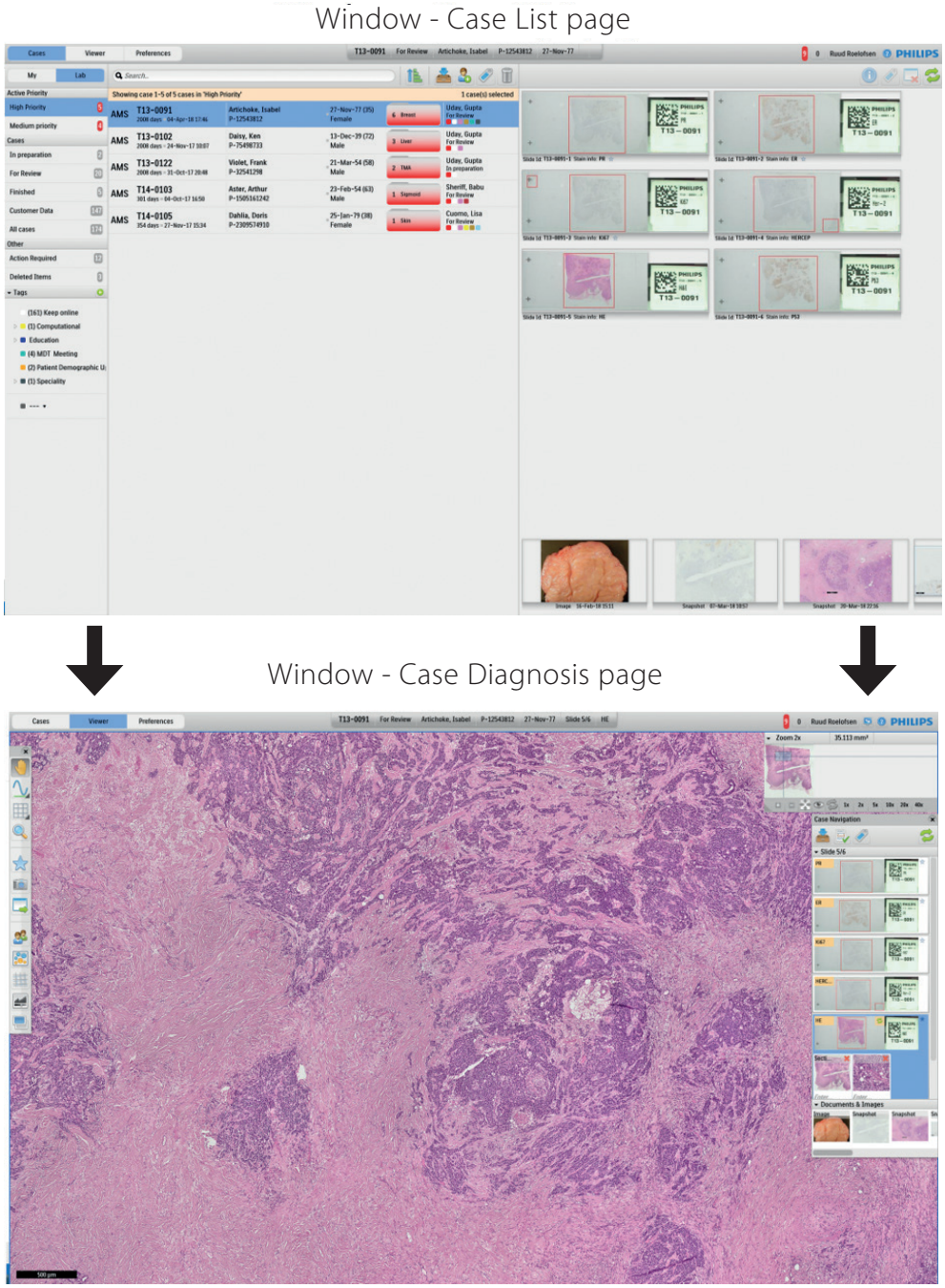


Figure 2. Example of the user interface of the workflow system used in this study. Left panel shows an overview of the cases assigned to a specific pathologist. Right panel shows the viewing window, allowing inspection of a scanned tissue section.

Sometimes, large time gaps between successive events in the log file were observed. It is unlikely that within these gaps the pathologist was studying the microscopic image, as also panning and zooming the case diagnosis window yield log file events. Most probably, the gaps represent activities other than diagnosing the case (e.g. talking to a colleague, phone calls, breaks). To compensate for the bias introduced by this phenomenon, an upper threshold had to be set to the time accepted between successive events. The threshold was established by inspection of histograms of time gaps for the participating pathologists. Please note that because in the conventional workflow the pathologists explicitly indicated beginning and end of reading sessions, no gap analysis was performed for that study arm.

RESULTS

Over a two months period, 1984 cases were read by 4 pathologists in the conventional workflow, and 1834 cases were read in the digital workflow by 5 pathologists (Table 1). Only 15 case categories had 20 or more cases for both conventional and digital reading and were included in the results, to enable calculating statistics with sufficient accuracy.

	Period	# pathologists	# cases	# case categories
Conventional	2 months	4	1984	125
Digital	2 months	5	1834	108

Table 1. Overview of cases assessed in this diagnostic reading time study in two arms: use of conventional optical microscopy versus digitized whole slide images.

Table 2 shows statistics on reading times using conventional microscopy for the most prevalent case categories (N>20). The median reading time per case ranged from 1.52 minutes for gall bladder to 9.73 minutes for prostate biopsies (prostate care program). Figure 3 shows the reading time distribution in boxplots, per case category (left boxes).

	conventional		digital	
	N	Mean	N	mean
appendix	21	2.88	26	1.87
Barrett / oesophagus	31	3.40	35	3.91
breast biopsy	59	9.62	79	4.90
colon biopsy	38	3.88	32	3.98
colon polyp excision	105	3.54	76	2.32
soft tissue resection benign	46	2.25	44	3.49
endometrium biopsy/curetting	82	3.11	68	3.77
gall bladder	53	2.62	37	1.74
lung biopsy	26	7.39	35	4.30
prostate biopsy	46	13.78	28	6.60
skin biopsy malignant	40	4.59	199	2.07
skin neoplasia	58	2.82	70	1.45
skin resection	537	3.52	461	2.52
stomach biopsy	62	5.34	69	3.70

Table 2. Comparison of mean reading times (in minutes) between conventional and digital reading of cases for the most prevalent case categories (N>20).

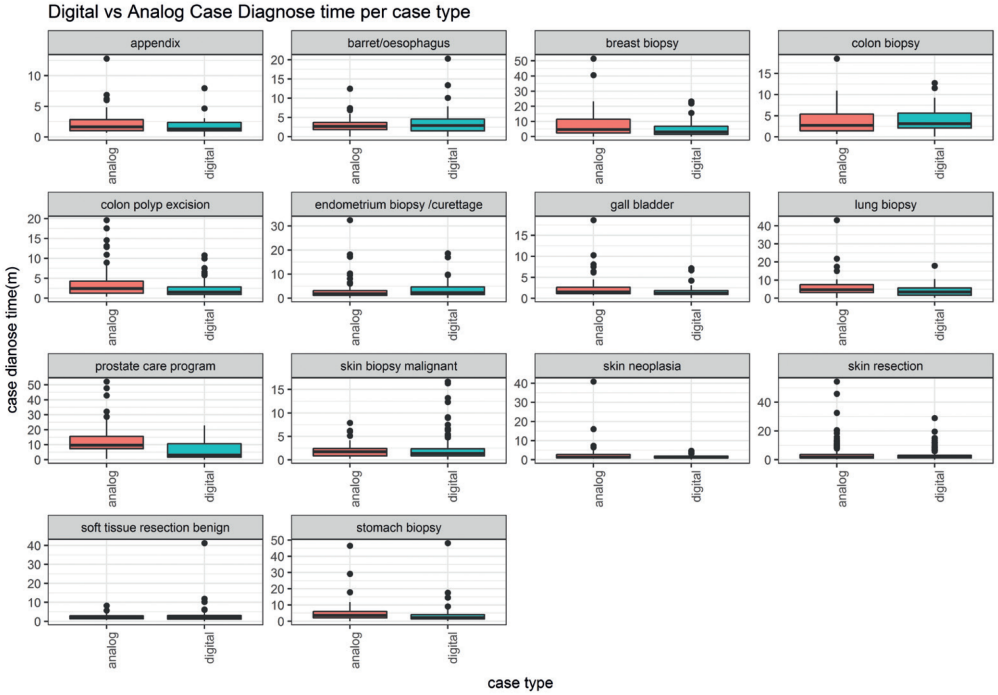


Figure 3. Boxplots of the distribution of time needed for diagnoses, per case category for the conventional (left) and digital (right) workflow. Data from the digital workflow are corrected for gaps, as indicated in the text.

Digital slide reading

To minimize the influence of short periods of time in which a pathologist was engaged in activities other than diagnostics, an upper limit was imposed on the duration of gaps in the log files. Figure 4 shows distributions of these gaps for the 5 individual pathologists. A total of 41 gaps (adding up to 3.5% of total reading time) even exceeded a duration of one hour. Figure 4 shows gaps below a duration of 1 hour. We observed a median gap time for the different pathologist of approximately 5 minutes. After consultation with the participating pathologists, this was found acceptable as a safe upper limit for gap duration to be taken into account in the analyses.

Table 2 lists reading time statistics for the digital workflow for the most prevalent case categories (N>20), ranging from a median of 1.25 minutes for skin neoplasias to 3.44 minutes for lung biopsies. The reading time distribution per case category is graphically shown in Figure 3 (right boxes).

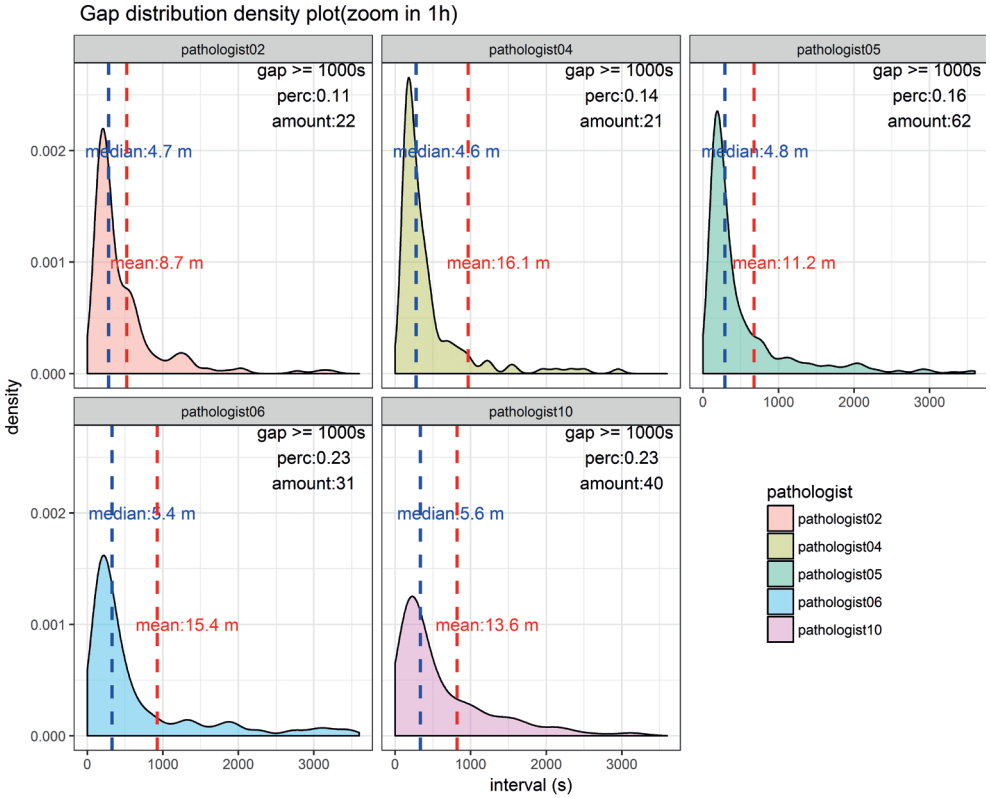


Figure 4. The distribution of gaps in the log files, for each pathologist individually.

Comparison between conventional and digital slide reading

Table 2 shows the differences between median reading time for the digital and conventional workflow for the most prevalent case categories. As can be seen, for most prevalent case categories the digital workflow required less time than the conventional. The largest time difference (both relative and absolute) was seen for prostate biopsies (6.64 min/68% time gain for the digital workflow).

By summing up the reading times of all cases, the total reading time was obtained for the conventional and digital workflows, and the average reading time was calculated for the 1984 conventionally read cases and the 1834 digital cases, respectively. This resulted in a relative time gain of 12.3% by digital reading.

DISCUSSION

In this study we assessed the effect on pathologist reading time when transitioning from a conventional workflow with a traditional optical microscope to a fully digitized workflow using whole slide images. The study was performed in a primary diagnostic setting and comprised >3800 cases total. We found that for most case categories the digital workflow required less reading time by the pathologist. Taking case mix into account, an average time gain of 12.3% was observed in favour of the digital workflow.

Results of published studies are not consistent in the effect of a digital workflow on pathologist reading time. An important explanation might be the fact that the benefits of a digital workflow are only fully exploited in an optimal situation [7]. Earlier studies often found that a digital workflow required more reading time by the pathologists [8,9]. It is likely that a less than optimal user interface combined with a lack of integration of the WSI viewer with the AP-LIS hampered a seamless operation, resulting in reduced efficiency for the digital workflow. In a later study, using an improved virtual microscope, no overall time difference was observed between digital and conventional workflows [10]. Although the digital workflow benefitted from the absence of physical loading and unloading of glass slides (making up 16% of time on the microscope), this potential time gain was lost again by significantly (even up to 6.6 times) more additional slide views in the digital workflow. The authors hypothesized that the additional slide views may be attributed to unfamiliarity with the relatively new technology [10].

A similar phenomenon was observed in a recent study on 510 cases [5]. Time required for diagnosis in a conventional versus digital workflow for three pathologists was studied in an experimental (i.e. non-clinical) set-up. It was concluded that the time difference between both modalities was negligible. However, the three pathologists were inexperienced in the digital workflow, probably resulting in longer reading times. This was clearly evidenced by the fact that during the cause of the study, case reading times in the digital pathology workflow significantly decreased [5].

The present study setup comprised a fully digitized and integrated primary diagnostic workflow which has been operational for several years. Therefore, the negative biases described

above will likely not be present. The digital pathologist workplace was optimized over a longer period of time prior to this study, comprising broad band network connections and big high resolution screens. Participating pathologists were experienced in digital slide reading. We consider this the optimal setup for this type of study, giving a realistic view on time required for diagnostic work in both study arms. Only in such an optimized setting, the potential improvements in pathologist efficiency are fully exploited. In a study comparing diagnostic time in a digital versus conventional workflow by a single pathologist, an overall time gain of 5.9% was observed in favour of digital slide reading (going from 97.8 to 92.05 minutes per session). Cases were primarily diagnosed in a digital workflow, which was repeated 6 months later in a conventional workflow. This time gain is 52% below that found in the present study. However, only the slide reviewing time was analysed, in contrast to the present study in which also time for case reporting was included resulting in the total time required for signing out a case. This process consisted of: workflow tasks (e.g. handling of individual slides, ordering of immunohistochemistry etc), slide review, and reporting. Stratman et al [4] performed a pathologist time and motion study, observing several pathologists during their routine work in a conventional (i.e. non-digital) setting. Time spent on these different subtasks was 36.0%, 34.6%, 13.4% and 16.0% for, respectively, slide review, reporting, workflow related tasks and other tasks (not further specified). They concluded that especially the workflow related tasks (13.4%) offered a window of opportunity for time gains by transitioning to a digital workflow, which seems to coincide well with the results of the present study.

In addition to the time gain found in the present study in case reading, significant pathologists' time may be saved in other tasks, such as preparation for tumor boards and consultations [1]. This was however beyond the scope of this study and depends on the storage parameters. Also, it is to be expected that algorithms based on artificial intelligence will significantly reduce pathologists' reading time, e.g. by pre-screening slides for presence of tumor [11].

Because the present study was entirely performed in a primary diagnostic setting, the two workflows inherently contained different cases. However, as there was no selection bias in including cases in one or the other study arm, the large number of cases in the two arms, and the largely comparable numbers per case category (see Table 2), we assume that no bias was introduced. Of note, to avoid any discussion about wash-out times that may vary considerably between individual pathologists, we deliberately decided to use other cases for conventional and digital pathology, but instead to use large numbers per case category.

One of the most important weaknesses in the present study is the requirement to extensively post-process the digital log-files and filter out gaps that most likely represent non-diagnostic activities. We chose to select a safe lower limit for the duration of gaps to exclude (5 mins), assuming that this will not positively bias the study results for the digital workflow.

In conclusion, over 10% pathologists' reading time can potentially be saved by transitioning to a fully integrated digital diagnostics workflow. The benefits of such a workflow are only optimally exploited in a fully integrated setup. This will help to build the business case to deal with the heavy investments required for such a fully digital workflow.

REFERENCES

1 Ho J, Ahlers SM, Stratman C, et al. Can digital pathology result in cost savings? A financial projection for digital pathology implementation at a large integrated health care organization. *J Pathol Inform*. 2014; 5: 33.

2 Mukhopadhyay S, Feldman MD, Abels E, et al. Whole Slide Imaging Versus Microscopy for Primary Diagnosis in Surgical Pathology: A Multicenter Blinded Randomized Noninferiority Study of 1992 Cases (Pivotal Study). *Am J Surg Pathol*. 2018; 42: 39--52.

3 Baidoshvili A, Bucur A, van Leeuwen J, van der Laak J, Kluin P, van Diest PJ. Evaluating the benefits of digital pathology implementation: Time savings in laboratory logistics. Histopathology 2018 Jun 20, Epub ahead of print.

4 Stratman C, Drogowski L, Ho J. Digital pathology in the clinical workflow. A Time and Motion Study [abstract]. San Diego, CA: Pathology Visions; 2011.

5 Mills AM, Gradecki SE, Horton BJ, et al. Diagnostic Efficiency in Digital Pathology: A Comparison of Optical Versus Digital Assessment in 510 Surgical Pathology Cases. *Am J Surg Pathol*. 2018; 42: 53--59.

6 Baidoshvili A. Whitepaper: How to go digital in pathology. October 2016. Available at https://thepathologist.com/fileadmin/issues/App_Notes/0016-022-app-note-How_to_ Go _Digital_in_Pathology.pdf. Accessed April 1, 2018.

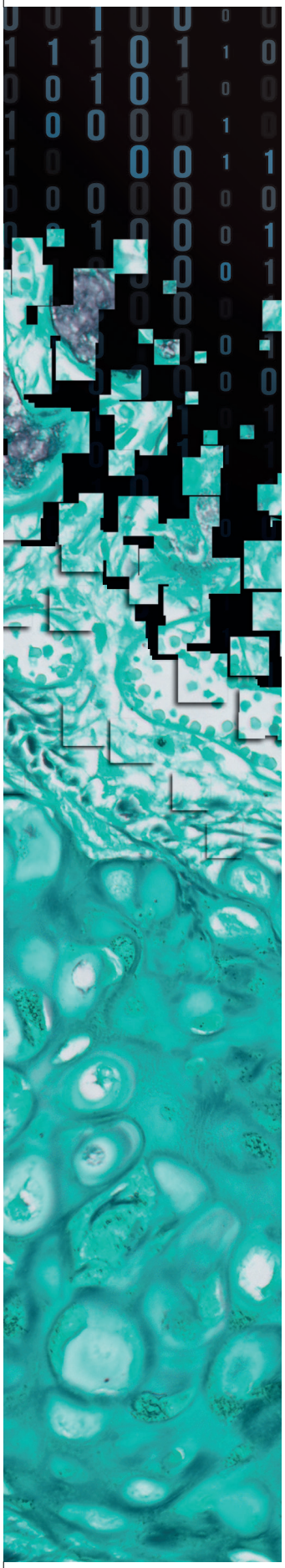
7 Vodovnik A. Diagnostic time in digital pathology: A comparative study on 400 cases. *J Pathol Inform*. 2016; 7: 4.

8 Treanor D, Quirke P. The Virtual Slide and Conventional Microscope - a direct comparison of their diagnostic efficiency. *J Pathol*. 2007; 213: 7a.

9 Velez N, Jukic D, Ho J. Evaluation of 2 whole-slide imaging applications in dermatopathology. *Hum Pathol*. 2008; 39: 1341--1349.

10 Randell R, Ruddle RA, Thomas RG, et al. Diagnosis of major cancer resection specimens with virtual slides: Impact of a novel digital pathology workstation. *Hum Pathol*. 2014; 45:2101-2106.

11 Ehteshami Bejnordi B, Veta M, van Diest PJ, et al. Diagnostic assessment of deep learning algorithms for detection of lymph node metastases in women with breast cancer. *JAMA*. 2017; 318: 2199-2210.



CHAPTER 6

Computer aided quantification of intratumoral stroma is an independent prognostic factor in rectal cancer

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Submitted

ABSTRACT

Purpose Tumor-stroma ratio (TSR) is an independent prognostic factor in colorectal cancer and other solid malignancies. The recent introduction of digital pathology in routine tissue diagnostics holds opportunities for automated TSR analysis. We investigated the potential of computer-aided quantification of intratumoral stroma in rectal cancer whole-slide images.

Methods Histological slides from 129 rectal adenocarcinoma patients were analyzed by two experts, who selected a suitable stroma hot-spot and visually assessed TSR. A semi-automatic method based on deep learning was trained to segment all relevant tissue types in rectal cancer histology and subsequently applied to the hot-spots provided by the experts. Patients were assigned to a 'stroma-high' or 'stroma-low' group by both TSR methods (visual and automated). This allowed for prognostic comparison between the two methods in terms of disease-specific and disease-free survival times.

Results With stroma-low as baseline, automated TSR was found prognostic independent of age, gender, pT-stage, lymph node status, tumor grade, and whether adjuvant therapy was given, both for DSS (hazard ratio (HR) = 2.48 (95% confidence interval (95% CI) 1.29-4.78)) and for DFS (HR = 2.05 (95% CI 1.11-3.78)). TSR-visual was not an independent prognostic factor in multivariate analysis.

Conclusions This work shows that deep learning based semi-automated TSR assessment in user-provided stroma hot-spots is prognostic for both disease-specific survival and disease-free survival, and may be a significant aid to human TSR assessment.

Keywords Rectal carcinoma, Tumor-stroma ratio, Prognosis, Computational pathology, Automated analysis, Deep learning

1 INTRODUCTION

In most solid malignancies, therapeutic decision making is primarily based on pathological staging of tumors. The traditional tumor, (lymph) node, metastasis (TNM) staging system [1] is routinely used to estimate patient prognosis and guide treatment worldwide. For certain tumor types, however, the TNM system lacks accuracy in assessing the metastatic potential of a tumor. For instance, TNM stage II colorectal cancer (CRC) comprises a heterogeneous group with diverse outcome [2]. As a result, the TNM stage is not informative for therapy planning of these patients, leading to both under- and over-treatment. Reliable new biomarkers are needed to guide personalized adjuvant treatment for these groups of patients.

A widely studied prognostic factor is the tumor-stroma ratio (TSR), expressing the relative amounts of tumor and intratumoral stroma. TSR is a straightforward measure which can be assessed by microscopic inspection of hematoxylin and eosin (H&E) stained tissue sections. TSR was shown to yield prognostic information in a range of solid malignancies, including breast cancer [3-5] and lung cancer [6, 7]. Generally, TSR is an independent prognostic factor, where a high content of intratumoral stroma is associated with poor prognosis. A number of previous studies showed promising results on the prognostic relevance of TSR in CRC [8-12]. Despite this evidence, there is no implementation in routine pathology reporting. This may be attributed to the variety in methodology and the lack of a standardized procedure for TSR assessment. Published studies propose visual assessment ('eyeballing'), systematic point counting, and the use of scanned (digitized) tissue sections (whole slide images; WSI). Although good inter-observer agreement was found in earlier studies [9, 11, 13], visual assessment of pathological quantitative features in general may suffer from reproducibility issues.

To facilitate an objective and standardized TSR assessment, image analysis and machine learning algorithms have been applied on H&E-stained sections of CRC before, however, these algorithms were applied to image regions extracted from WSI. Computer-aided tumor and stroma quantification was proposed based on automated tissue segmentation in H&E-stained sections using a combination of hand-crafted features and machine learning [14]. Furthermore, TSR was computed via automated point counting in H&E-stained images [15]. Similar image analysis techniques based on classical machine learning were applied to tissue microarrays stained with epidermal growth factor receptor (EGFR) immunohistochemistry [16, 17]. A new branch of machine learning algorithms, so-called deep learning algorithms, have recently entered the field of computational pathology and shown promise for automating certain tasks in histopathology. Detection of sentinel lymph node metastases [18] and of cancer in prostate biopsies [19] could successfully be performed using convolutional neural networks (CNN), a specific type of deep learning. We recently showed [20] that a deep learning based algorithm can distinguish between 9 different types of tissue in CRC WSI with an overall accuracy of 93.8%.

The present study aims to leverage our previously developed CNN for automated TSR assessment in the CRC sub-class of rectal adenocarcinomas. Only a limited number of studies have been published on TSR for rectal cancers and in a sub-analysis ($n = 43$) by West *et al.* [12] its

prognostic value could not be confirmed. Work by Scheer *et al*. [8] recently showed that TSR has potential as a prognostic factor for survival in surgically treated rectal cancer patients, however, TSR was only found to be and independent prognosticator in lymph node metastasis negative cases. The performance of the automated TSR system described here will be compared with data from human experts and its prognostic value will be evaluated in terms of disease-specific and disease-free survival times.

2 MATERIALS AND METHODS

2.1 Patients

An existing cohort of 154 patients [8] with rectal adenocarcinoma stages I-III was used. All patients received curative surgery in the period 1996-2006 at the Medisch Spectrum Twente hospital (The Netherlands). No patient was neoadjuvantly treated with radiotherapy and/or chemotherapy or died within 30 days after surgery. At the time of surgery, no patient had known distant metastases, inflammatory bowel disease, hereditary nonpolyposis colorectal cancer (HNPCC) or other/earlier cancers. Histopathological data was obtained from the Laboratory for Pathology Eastern Netherlands (LabPON). Clinical data was obtained from the Medisch Spectrum Twente hospital and the Netherlands Comprehensive Cancer Organization (IKNL). Collected clinicopathological data included tumor grade (differentiation), depth of invasion (pT) and lymph node involvement (pN) according to Union Internationale Contre le Cancer/American Joint Cancer Committee (UICC/AJCC) TNM staging system [1]. Data regarding adjuvant therapy and local or distant recurrence was also available.

2.2 Tissue slide preparation and scanning

Standard formalin fixed paraffin embedded tissue sections were cut and stained with hematoxylin and eosin (H&E) for routine diagnostic purposes. For the present study, a single slide per patient was selected which contained the most invasive part of the tumor and was used in diagnostics to assess the tumor pT-status. Slides were scanned at ×200 total magnification (tissue level pixel size 0.455 µm/pixel) using a Hamamatsu NanoZoomer 2.0-HT (C9600-13) scanner (Herrsching, Germany).

2.3 Visual estimation of intratumoral stroma

Two observers (GvP, WM; both > 10 years of experience with TSR scoring) independently scored the glass slides using a conventional light microscope according to a previously published protocol for TSR assessment [9, 10]. Briefly, the procedure consisted of: 1) coarse localization of the tissue area with the highest intratumoral stroma content at low microscope magnification, and 2) selection of one field of view at x100 total magnification and perform visual estimation of the tumor-stroma ratio (TSR-visual) in the selected circular region. Ideally, the selected region should meet the following criteria: high intratumoral stroma content (predominantly found at

the invasive margin of a tumor); presence of tumor cells at all borders of the field of view; no large quantities of muscle, mucus, necrosis or large vessels; and no tears or tissue retraction artefacts. As much as possible, the region with the highest stroma content (stroma hot-spot) was selected that met all the above requirements. TSR-visual was estimated by both observers independently, using 10% increments. As a result of the specific microscope and lenses used, the specimen-level diameter of the circular region was 1.8 mm at x100 magnification. There is a lot of variation among published studies concerning used TSR procedures (e.g. major differences in the location and size of the assessed tissue regions as well as what was actually measured: relative tumor or stroma content). For clarity, in this study the tumor-stroma ratio was defined as TSR = 100% × [intratumoral stroma area] / [tumor area + intratumoral stroma area]. Lumen, tears and other tissue types in the selected circular region were excluded during visual estimation. Lastly, the tissue region considered most suitable for TSR assessment was identified during a consensus meeting between the two observers in which 1) a binary TSR consensus score was determined: 'stroma-low' or 'stroma-high', and 2), the center of the stroma hot-spot was marked on the glass slide.

2.4 Automated computation of intratumoral stroma

To study the value of applying a deep learning algorithm for automated TSR assessment (TSR-auto), a CNN was developed similar to a previously published algorithm [20]. The CNN performs tissue segmentation (i.e. subdivision of tissue areas) of H&E-stained rectal cancer WSIs into nine different classes: tumor, intratumoral stroma, necrosis, muscle, healthy epithelium, fatty tissue, lymphocytes, mucus and erythrocytes. The CNN was trained using manually annotated regions in 74 WSI taken from the cohort used in this study. Regions to annotate were selected for covering tissue variety across WSI, rather than producing exhaustive annotations on a small number of WSI. Annotations were produced by a pathology researcher (OG) and a medical student, and were checked and corrected when deemed necessary by an experienced pathologist (AB). Unlike Ciompi *et al.* [20] here we used patches of 256x256 pixels for classification, which experimentally showed to improve performance and produce a smoother segmentation map (data not shown). Performance of the system was assessed by segmenting all WSIs in the dataset in a five-fold cross validation fashion (at WSI level) and evaluating accuracy in all annotated regions.

To enable comparison, the CNN based TSR-auto was computed in the same circular region that was selected by the observers in the consensus meeting, where TSR-visual was assessed. The image data in the circular region (with diameter 1.8mm) was extracted from each WSI and processed further by the CNN described above (Fig. 1). Segmentation of a WSI into nine different tissue classes enabled in- and exclusion of specific tissue types comparable to the visual assessment procedure. The used definition of TSR-auto is similar to TSR-visual, expressing the area consisting of stroma as a percentage of the area occupied by both tumor and stroma.

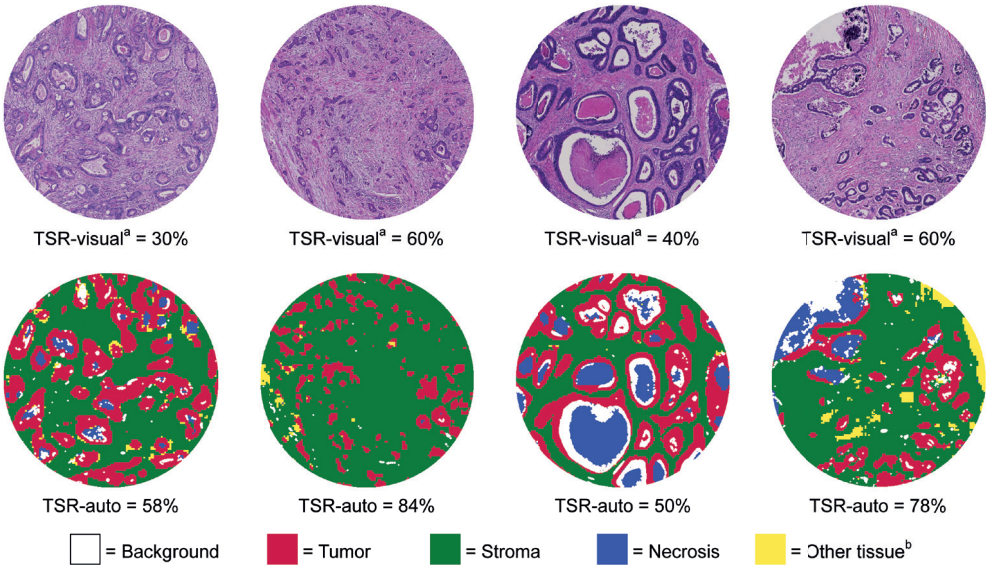


Fig. 1 Top: Stroma hot-spots selected by the observers for the assessment of TSR-visual[a]. Bottom: The same regions with tissues segmented by the CNN for the calculation of TSR-auto. [a]Observer consensus; [b]Other tissue includes classes: muscle, healthy epithelium, fatty tissue, lymphocytes, mucus and erythrocytes

2.5 Statistical analyses

In this study, TSR-visual and TSR-auto were compared as prognostic factors in rectal cancer. Statistical analyses were performed using IBM SPSS software v24.0 (Armonk, NY, USA). The intraclass correlation coefficient (ICC) was used to determine the correlation between TSR assessed by two observers and by the automated method. To investigate a possible relationship between clinicopathological variables and the numerical values of TSR-visual and TSR-auto, Mann–Whitney U and Kruskal–Wallis tests were performed for two- and multi-class variables, respectively. For further statistical analysis, TSR-visual and TSR-auto were dichotomized, subdividing patients into two groups: 'stroma-low' and 'stroma-high'. Dichotomization of TSR-visual was performed based on a cut-off value previously established10 on 63 colon cancer cases: stroma-high = TSR-visual > 50% and stroma-low = TSR-visual ≤ 50%. In this study, we analyzed results for two different cut-off values for TSR-auto since the optimal cut-off value for the automated approach is not yet established. One method of dichotomization used the '50% stroma cut-off', similar to TSR-visual, referred to as TSR-auto(50%), and the other dichotomization method was based on the median value for all measured TSR-auto values, referred to as TSR-auto(median), yielding equal numbers of patients in stroma-low and stroma-high groups.

Inter-observer agreements were calculated using Cohen's Kappa (κ) on the dichotomized TSR values. Kaplan-Meier survival analyses were performed and log-rank statistics were used to test differences in both disease-specific survival (DSS) and disease-free survival (DFS) distributions.

DSS was defined as the time between the date of surgery and the date of death attributable to rectal adenocarcinoma. For DFS, the date of the first event of cancer recurrence was used, which could be loco-regional or a distant metastasis. In case no event occurred, the time period until the last date of follow-up was used in the survival analyses. Finally, both uni- and multivariate analyses were performed for TSR-visual and TSR-auto using the Cox proportional hazards model. Probability values < 0.05 (2-sided) were considered statistically significant.

3 RESULTS

3.1 Clinicopathological data

Of 154 patients projected for inclusion in this study, twelve cases with mucinous carcinoma were excluded as these tumors have largely different TSR values. Twelve other cases were excluded because, at the time of writing, the required slides or data were unavailable. One case was excluded because the corresponding tissue slide did not contain invasive carcinoma.

The median follow-up time for the remaining 129 patients used in the present study was 5.6 years (interquartile range 2.3 – 8.3). The median age of the patients at time of surgery was 67 years (interquartile range 59 – 74). Further clinicopathological data can be found in Table 1. There was no significant correlation between the clinicopathological variables and TSR-visual or TSR-auto ($p > 0.05$).

3.2 Performance deep learning system

Measures of sensitivity and specificity per tissue type as well as overall accuracy were assessed for the automatic method by pixel-wise comparison of predicted labels with ground truth labels in manually annotated regions. Overall accuracy was 94.6%, which shows improvement on what was reported by Ciompi *et al.* [20]. Values of per-class sensitivity and specificity are reported in Table 2.

3.3 Inter-observer and computer-observer agreement

The ICC between the two observers for the assessment of TSR was 0.736 (95% confidence interval (95% CI) 0.646-0.806). The co-occurrence of TSR scores assessed by the two observers is depicted in Fig. 2. The ICC's between TSR-auto and TSR-visual were 0.475 (95% CI 0.330-0.598) and 0.411 (95% CI 0.257-0.545) for observers 1 and 2, respectively.

A moderate agreement between the two observers ($\kappa = 0.578$) was found after dichotomizing TSR-visual on the basis of the 50% cut-off as described above. Using the identical cut-off for TSR-auto, we observed only a fair agreement between TSR-visual and TSR-auto ($\kappa = 0.239$). Agreement improved considerably ($\kappa = 0.521$) when the median was used as cut-off for TSR-auto, resulting in: stroma-low = TSR-auto ≤ 65.47% and stroma-high = TSR-auto > 65.47%. Patients assigned to stroma-low or stroma-high groups by the observers and the automatic method are detailed in Tables 3, 4 and 5.

	Total	TSR-visual[a]		TSR-auto(50%)		TSR-auto(median)	
		Stroma-low	Stroma-high	Stroma-low	Stroma-high	Stroma-low	Stroma-high
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Gender							
Female	43 (34)	30 (34)	13 (31)	11 (35)	32 (33)	22 (34)	21 (32)
Male	86 (67)	57 (66)	29 (69)	20 (65)	66 (67)	42 (66)	44 (68)
T-status							
pT1	4 (3)	4 (5)	0 (0)	2 (6)	2 (2)	3 (5)	1 (2)
pT2	40 (31)	29 (33)	11 (26)	5 (16)	35 (36)	18 (28)	22 (34)
pT3	79 (61)	51 (59)	28 (67)	24 (77)	55 (56)	42 (66)	37 (57)
pT4	6 (5)	3 (3)	3 (7)	0 (0)	6 (6)	1 (2)	5 (8)
N-status							
pN0	78 (60)	54 (62)	24 (57)	22 (71)	56 (57)	43 (67)	35 (54)
pN1	33 (26)	23 (26)	10 (24)	6 (19)	27 (28)	13 (20)	20 (31)
pN2	18 (14)	10 (11)	8 (19)	3 (10)	15 (15)	8 (13)	10 (15)
Stage							
I	33 (26)	26 (30)	7 (17)	6 (19)	27 (28)	18 (28)	15 (23)
II	45 (35)	28 (32)	17 (40)	16 (52)	29 (30)	25 (39)	20 (31)
III	51 (40)	33 (38)	18 (43)	9 (29)	42 (43)	21 (33)	30 (46)
Tumor diff. grade							
Well	3 (2)	2 (2)	1 (2)	1 (3)	2 (2)	1 (2)	2 (3)
Moderate	112 (87)	73 (84)	39 (93)	28 (90)	84 (86)	55 (86)	57 (88)
Poor	14 (11)	12 (14)	2 (5)	2 (6)	12 (12)	8 (13)	6 (9)
Surgery type							
APR	62 (48)	39 (45)	23 (55)	10 (32)	52 (53)	26 (41)	36 (55)
LAR	49 (38)	37 (43)	12 (29)	17 (55)	32 (33)	29 (45)	20 (31)
Hartmann	18 (14)	11 (13)	7 (17)	4 (13)	14 (14)	9 (14)	9 (14)
Adjuvant treatment							
None	86 (67)	59 (68)	27 (64)	24 (77)	62 (63)	45 (70)	41 (63)
Radiotherapy	43 (33)	28 (32)	15 (36)	7 (23)	36 (37)	19 (30)	24 (37)
Chemoradioth.[b]	5 (4)	3 (3)	2 (5)	1 (3)	4 (4)	2 (3)	3 (5)

Mann–Whitney U and Kruskal–Wallis tests showed no significant correlation ($p > 0.05$) between the listed variables and TSR-visual or TSR-auto. [a]Observer consensus; [b]Chemoradiotherapy. LAR: Low anterior resection; APR: Abdominoperineal resection; pT: Pathological tumor status; pN: Pathological nodal status.

Table 1 Clinicopathological data for 129 rectal cancer patients in relation to TSR-visuala and TSR-auto.

	Tumor	Stroma	Necrosis	Muscle	Healthy epith.	Fat	Mucus	Lympho-cytes	Blood
Sensitivity	91.1%	91.7%	90.8%	95.5%	94.1%	98.1%	96.4%	98.4%	97.9%
Specificity	99.4%	97.7%	99.6%	99.6%	99.5%	99.9%	98.7%	99.6%	99.9%

CNN: Convolutional Neural Network; Healthy epi.: Healthy epithelium

Table 2 Quantitative performance of the CNN at pixel classification per tissue class

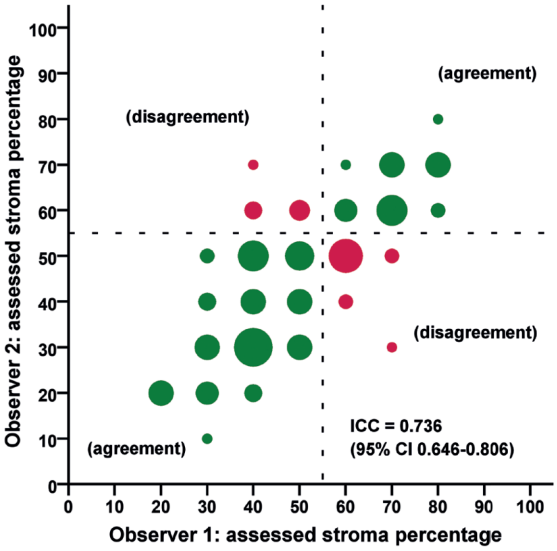


Fig. 2 Scatter plot of assessed stroma percentages in 129 patients for Observer 1 and Observer 2. The co-occurrence of assessed percentages is indicated by circles with areas proportional to the amount of patients scored with the corresponding TSR value. The dashed lines represent the boundary between stroma-low and stroma-high cases according to the cut-off value determined in Mesker *et al*. [10] Green circles indicate cases where the observers agreed (105 in total) and red circles indicate disagreement (24 in total)

	$\kappa = 0.578$	Observer 2		
		Stroma-low	Stroma-high	Total
Observer 1	Stroma-low	75	8	83
	Stroma-high	16	30	46
	Total	91	38	129

Table 3 Cross-tabulation of Observer 1 versus Observer 2 after dichotomisation

	κ = 0.239	TSR-auto (50%)		
		Stroma-low	Stroma-high	Total
TSR-Visual (consensus)	Stroma-low	30	57	87
	Stroma-high	1	41	42
	Total	31	98	129

Table 4 Cross-tabulation of TSR-visual (consensus) versus TSR-auto (50%) after dichotomization

	κ = 0.521	TSR-auto(median)
		Stroma-low
TSR-visual (consensus)	Stroma-low	60
	Stroma-high	4
	Total	64

Table 5 Cross-tabulation of TSR-visual (consensus) versus TSR-auto(median) after dichotomisation

3.4 Survival analyses

Survival analysis generally showed a worse outcome for stroma-high patients compared to stroma-low patients (Fig. 3), independent of the method of TSR assessment (visual versus automated). For TSR-visual, the 5-year survival rates for stroma-low versus stroma-high cases were 71.0% versus 58.8% for DSS and 65.6% versus 49.1% for DFS. For TSR-auto(50%), the 5-year survival rates for stroma-low versus stroma-high cases, were 86.6% versus 60.7% for DSS and 76.8% versus 54.9% for DFS. For TSR-auto(median), the 5-year survival rates for stroma-low versus stroma-high cases, were 76.1% versus 58.4% for DSS and 70.0% versus 50.7% for DFS.

For TSR-visual, a significantly lower DSS was seen in the stroma-high group compared to the stroma-low group (p = 0.042) but not for DFS (p = 0.182). Similarly, for TSR-auto(50%) this difference was significant for DSS (p = 0.018) but not for DFS (p = 0.066). For TSR-auto(median), both DSS and DFS were significantly lower in the stroma-high group compared to the stroma-low group (p = 0.007 and p = 0.021, respectively). After stratification for TNM stage, stroma-high was also associated with worse survival in stage II rectal cancer patients (n = 45), but this result was only significant for TSR-auto(median) (DSS p = 0.003 and DFS p = 0.015).

Hazard ratios (HR) and 95% CIs were determined for both DSS and DFS (Tables 6 and 7). In univariate analysis, all methods for TSR assessment were found prognostic for DSS: TSR-visual HR = 1.83 (95% CI 1.01-3.30); TSR-auto(50%) HR = 2.71 (95% CI 1.14-6.40); and TSR-auto(median) HR = 2.31 (95% CI 1.24-4.30). For DFS, only TSR-auto(median) was found prognostic with HR = 1.96 (95% CI 1.10-3.51). After stratification for TNM stage, only TSR-auto(median) was found prognostic for stage II rectal cancer patients both for DSS (univariate HR = 4.13 (95% CI 1.53-11.16)) and DFS (univariate HR = 3.05 (95% CI 1.19-7.81)).

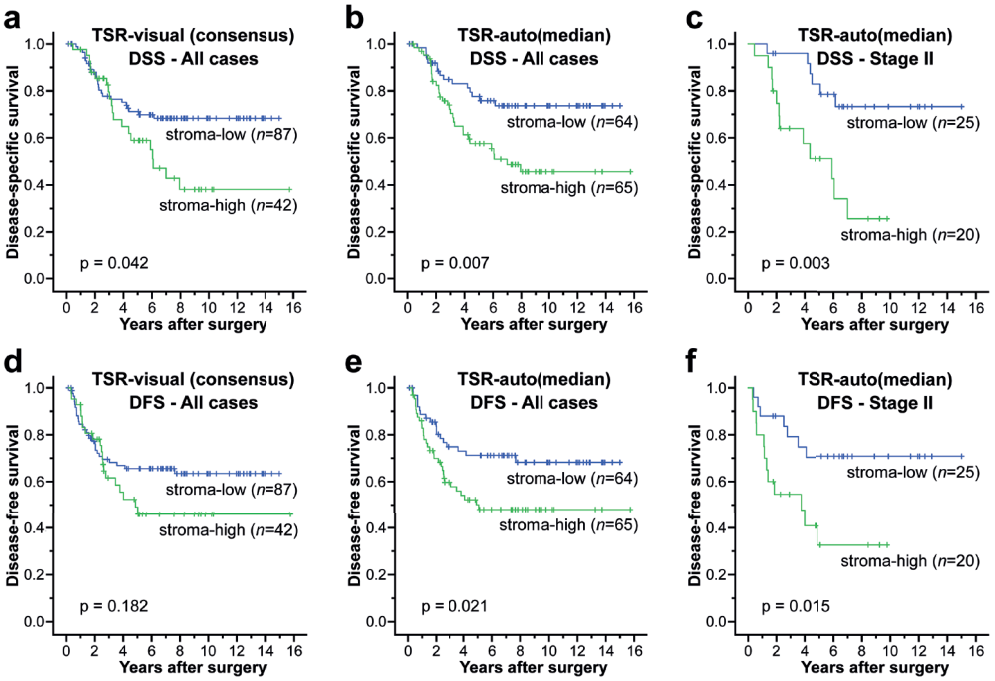


Fig. 3 Kaplan-Meier curves for disease-specific survival (top row) and disease-free survival (bottom row) of stroma-low versus stroma-high patients. Results based on all patients ($n = 129$) are shown for TSR-visual (**a, d**) and TSR-auto(median) (**b, e**). Results for patients with stage II rectal cancer ($n = 45$) are shown for TSR-auto(median) only (**c, f**). Log-rank test p-values are shown in the graphs

	Univariate		Multivariate					
			TSR-visual[a]		TSR-auto (50%)		TSR-auto (median)	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Age[b]	1.01	0.98-1.04	1.01	0.98-1.04	1.00	0.98-1.03	1.01	0.98-1.04
Gender								
Female	1.00		1.00		1.00		1.00	
Male	1.16	0.61-2.17	1.64	0.83-3.24	1.67	0.83-3.34	1.62	0.83-3.18
T-status[c]								
pT1–pT2	1.00		1.00		1.00		1.00	
pT3–pT4	**4.52**	**1.91-10.72**	**3.75**	**1.56-9.03**	**4.80**	**1.96-11.75**	**4.48**	**1.84-10.91**
LN metastases[d]								
No	1.00		1.00		1.00		1.00	
Yes	**2.93**	**1.35-6.32**	1.64	0.53-5.08	1.43	0.45-4.57	1.26	0.42-3.76
Tumor grade[e]								
Well–Moderate	1.00		1.00		1.00		1.00	
Poor	**2.86**	**1.32-6.20**	**2.87**	**1.24-6.69**	2.29	0.96-5.47	**2.63**	**1.14-6.08**
Adjuvant therapy								
No	1.00		1.00		1.00		1.00	
Yes	**2.31**	**1.28-4.15**	1.28	0.39-4.17	1.25	0.37-4.22	1.42	0.44-4.54
TSR-visual[a]								
Stroma-low	1.00		1.00					
Stroma-high	**1.83**	**1.01-3.30**	1.76	0.93-3.34				
TSR-auto(50%)								
Stroma-low	1.00				1.00			
Stroma-high	**2.71**	**1.14-6.40**			**3.11**	**1.26-7.70**		
TSR-auto(median)								
Stroma-low	1.00						1.00	
Stroma-high	**2.31**	**1.24-4.30**					**2.48**	**1.29-4.78**

[a]Observer consensus; [b]Age was used as a continuous variable; [c]Due to low numbers, pT1 (n = 4) and pT2 cases were grouped together as well as pT3 and pT4 (n = 6) cases; [d]Lymph node metastases; [e]Due to low numbers, cases with well (n = 3) and moderately differentiated tumors were grouped together.

Table 6 Uni- and multivariate Cox regression analysis for disease-specific survival

	Univariate		Multivariate					
			TSR-visual[a]		TSR-auto(50%)		TSR-auto(median)	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Age[b]	1.00	0.97-1.02	1.00	0.97-1.02	0.99	0.96-1.02	0.99	0.97-1.02
Gender								
Female	1.00		1.00		1.00		1.00	
Male	1.35	0.73-2.52	1.87	0.96-3.63	1.80	0.92-3.52	1.81	0.94-3.49
T-status[c]								
pT1–pT2	1.00		1.00		1.00		1.00	
pT3–pT4	**4.09**	**1.83-9.12**	**3.52**	**1.55-8.01**	**4.33**	**1.87-10.04**	**4.11**	**1.78-9.48**
LN metastases[d]								
No	1.00		1.00		1.00		1.00	
Yes	**2.43**	**1.38-4.28**	1.89	0.68-5.25	1.78	0.63-5.00	1.55	0.58-4.19
Tumor grade[e]								
Well–Moderate	1.00		1.00		1.00		1.00	
Poor	**2.24**	**1.05-4.80**	2.02	0.88-4.62	1.65	0.70-3.87	1.78	0.78-4.06
Adjuvant therapy								
No	1.00		1.00		1.00		1.00	
Yes	**2.22**	**1.26-3.89**	1.07	0.37-3.10	0.98	0.33-2.90	1.11	0.39-3.15
TSR-visual[a]								
Stroma-low	1.00		1.00					
Stroma-high	1.47	0.83-2.61	1.42	0.77-2.61				
TSR-auto(50%)								
Stroma-low	1.00				1.00			
Stroma-high	2.01	0.94-4.29			**2.39**	**1.07-5.38**		
TSR-auto(median)								
Stroma-low	1.00						1.00	
Stroma-high	**1.96**	**1.10-3.51**					**2.05**	**1.11-3.78**

[a]Observer consensus; [b]Age was used as a continuous variable; [c]Due to low numbers, pT1 (***n*** = 4) and pT2 cases were grouped together as well as pT3 and pT4 (***n*** = 6) cases; [d]Lymph node metastases; [e]Due to low numbers, cases with well (***n*** = 3) and moderately differentiated tumors were grouped together.

Table 7 Uni- and multivariate Cox regression analysis for disease-free survival

In multivariate analysis, automated TSR assessment was found prognostic independent of age, gender, pT-stage, lymph node status, tumor grade, and whether adjuvant therapy was given, both for DSS: TSR-auto(50%) HR = 3.11 (95% CI 1.26-7.70) and TSR-auto(median) HR = 2.48 (95% CI 1.29-4.78), and for DFS: TSR-auto(50%) (HR = 2.39 (95% CI 1.07-5.38)) and TSR-auto(median) (HR = 2.05 (95% CI 1.11-3.78)). TSR-visual was not an independent prognostic factor.

4 DISCUSSION

For different cancer types, TSR has been shown to yield prognostic information. Human visual assessment of TSR requires training, and may be difficult for cases close to the decision threshold of 50%. The present study showed that specifically for rectal adenocarcinoma the observer agreement is only moderate. Recent advances in slide scanning technology and machine learning have opened new possibilities for computerized assessment of TSR. To the best of our knowledge, the present study showed for the first time that TSR can reliably be assessed by an automatic deep learning algorithm. The agreement of the automated system (using median cut-off) with the human consensus (kappa = 0.521) was comparable to the inter-observer agreement (kappa = 0.578). The TSR assessed in this manner appeared to be a strong independent prognostic factor both for DSS and DFS in rectal adenocarcinoma. The prognostic value of the automated TSR was comparable to that assessed in consensus by two experienced human observers for DSS. For DFS, only the automatically assessed TSR was significantly associated with outcome.

Interestingly, automated TSR (using the median as cut-off) showed prognostic value for TNM stage II patients. Clinically, this is a subgroup of patients for which post-operative treatment is still under debate and more research is needed [21, 22]. TSR can potentially help to direct this discussion and add information for a more personalized treatment of this patient category.

In a recent study, Scheer *et al.* [8] analyzed TSR on the same cohort of patients as used in the present study. However, rather than a hot-spot measure, the authors applied a scoring procedure in which an average TSR was assessed based on the entire tumor area in a slide. Also, they defined TSR as the carcinoma percentage (CP) and the estimated percentages were grouped using three categories (low-CP, intermediate-CP and high-CP). In univariate survival analysis, CP was found to be prognostic for DSS and DFS. With CP-high as baseline and after correction for age, grading, pathological T-stage, and adjuvant treatment, CP-intermediate was correlated with worse DSS and DFS, however, this result was only found for the subset of lymph node metastasis negative cases (n= 94). In the present study, the prognostic value of TSR remained intact for the entire cohort of patients after correction for clinicopathological variables, including lymph node status. The most probable cause for this difference is the TSR scoring method. In the present study we selected to follow a more widely accepted scoring system, which appears to outperform a method where the overall tumor area is scored by averaging.

The results of our observer study indicate that TSR obtained by human visual estimation is a prognostic factor of DSS (although not reaching statistical significance when correcting for other clinicopathological features) but not of DFS. Furthermore, only a moderate agreement was shown between human observers. These results are in contrast with previous studies [9, 10, 13] on TSR assessment on colon cancer. This might be explained by the fact that compared to colon, the rectum bowel wall has a thicker muscle layer and in some cases it might be difficult to distinguish between stromal tissue and smooth muscle cells, especially with darker H&E-stained slides. Muscle tissue, which should be excluded from scoring, may therefore be interpreted as stromal tissue by one observer and not by the other. Furthermore, as shown in Fig. 2, most

discrepancies (15/24 cases) are found around the cut-off point of 50%. Especially for these cases, computer-aided TSR assessment could be very useful.

For the automated method two different stroma cut-off values have been investigated in this study: the value used for the human visual estimation (50%), and the median of measured TSR-auto values. We found comparable results for the two cut-offs, with slightly higher HR for the 50% cut-off. However, as in general automated assessment of TSR yields higher values than manual assessment, the 50% cut-off corresponded much less to manual assessment than the median cut-off (as is reflected in the kappa values). Because TSR-auto gives higher stroma percentages, a cut-off which is optimized for this procedure may be required. The optimal cut-off value for TSR-auto should be further investigated and validated in an independent cohort.

It is worth noting that one of the inclusion criteria for patient selection in this study was the absence of neoadjuvant treatment. The reason for this design choice was that both chemotherapy and radiotherapy modify the tissue architecture and may hamper the computation of TSR. Our method can therefore aid clinicians in selecting the right treatment options for rectal cancer patients who did not receive preoperative (chemo)radiotherapy. Furthermore, given the fact that the colon and the rectum are parts of the same continuous organ and have a similar histological appearance, the presented deep learning algorithm has the potential to be successfully applied to the analysis of colon cancer as well.

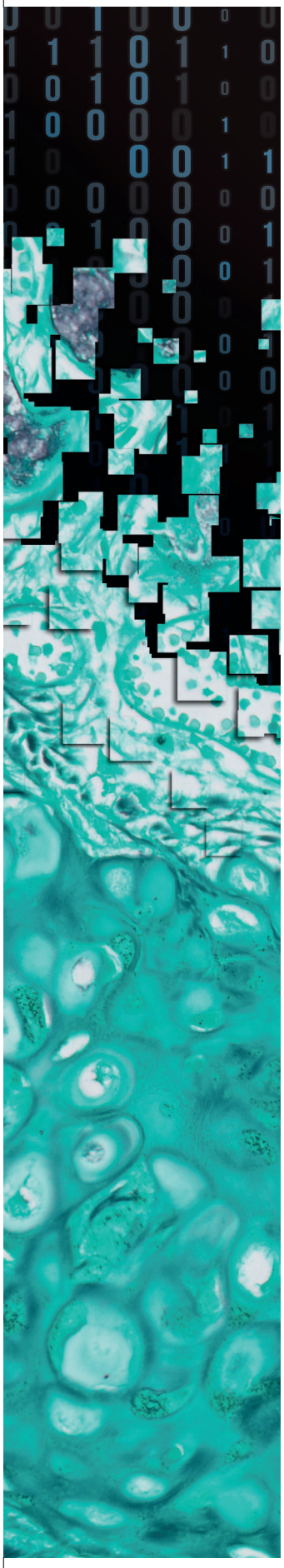
The deep learning based approach proposed in this work needs the position of a user-provided stroma hot-spot as input in order to assess TSR. As such, human input is still required, making the method only semi-automatic. It is worth noting that in Ciompi *et al.* [20] a similar computer model as was used in this work has shown high performance at segmenting several tissue types in rectal cancer at whole-slide image level, i.e., beyond the limited area of the selected hot-spot. As a consequence, this method has the potential to be used to quantify TSR both at whole-tumor level and at whole-slide image level. Such an approach would overcome the need for a user-provided stroma hot-spot and therefore allow investigating TSR at very large scale via fully-automatic computation.

The objectiveness of a deep learning based method, which allows obtaining accurate and reproducible quantification of TSR, has the potential to pave the way to the implementation of TSR in clinical practice.

REFERENCES

1. L. H. Sobin and I. D. Fleming, TNM classification of malignant tumors, Fifth edition. Cancer. 80, 1803–1804 (1997)
2. H. J. Schmoll, E. van Cutsem, A. Stein, V. Valentini, B. Glimelius, K. Haustermans, B. Nordlinger, C. J. van de Velde, J. Balmana, J. Regula, I. D. Nagtegaal, R. G. Beets-Tan, D. Arnold, F. Ciardiello, P. Hoff, D. Kerr, C. H. Köhne, R. Labianca, T. Price, W. Scheithauer, A. Sobrero, J. Tabernero, D. Aderka, S. Barroso, G. Bodoky, J. Y. Douillard, H. El ghazaly, J. Gallardo, A. Garin, R. Glynne-jones, K. Jordan, A. Meshcheryakov, D. Papamichail, P. Pfeiffer, I. Souglakos, S. Turhal, and A. Cervantes, ESMO Consensus Guidelines for management of patients with colon and rectal cancer. A personalized approach to clinical decision making. Ann. Oncol. 23, 2479–2516 (2012)
3. F. J. A. Gujam, J. Edwards, Z. M. A. Mohammed, J. J. Going, and D. C. McMillan, The relationship between the tumour stroma percentage, clinicopathological characteristics and outcome in patients with operable ductal breast cancer. Br. J. Cancer. 111, 157–165 (2014)
4. A. M. Moorman, R. Vink, H. J. Heijmans, J. Van Der Palen, and E. A. Kouwenhoven, The prognostic value of tumour-stroma ratio in triple-negative breast cancer. Eur. J. Surg. Oncol. 38, 307–313 (2012)
5. T. Roeke, M. Sobral-Leite, T. J. A. Dekker, J. Wesseling, V. T. H. B. M. Smit, R. A. E. M. Tollenaar, M. K. Schmidt, and W. E. Mesker, The prognostic value of the tumour-stroma ratio in primary operable invasive cancer of the breast: a validation study. Breast Cancer Res. Treat. 166, 435–445 (2017)
6. Z. Wang, H. Liu, R. Zhao, H. Zhang, C. Liu, and Y. Song, Tumor-stroma ratio is an independent prognostic factor of non-small cell lung cancer. Chinese J. lung cancer. 16, 191–196 (2013)
7. T. Zhang, J. Xu, H. Shen, W. Dong, Y. Ni, and J. Du, Tumor-stroma ratio is an independent predictor for survival in NSCLC. Int. J. Clin. Exp. Pathol. 8, 11348–11355 (2015)
8. R. Scheer, A. Baidoshvili, S. Zoidze, M. A. Elferink, A. E. Berkel, J. M. Klaase, and P. J. van Diest, Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: A retrospective cohort study. World J. Gastrointest. Oncol. 9, 466–474 (2017)
9. A. Huijbers, R. A. E. M. Tollenaar, G. W. van Pelt, E. C. M. Zeestraten, S. Dutton, C. C. McConkey, E. Domingo, V. T. H. B. M. Smit, R. Midgley, B. F. Warren, E. C. Johnstone, D. J. Kerr, and W. E. Mesker, The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: Validation in the victor trial. Ann. Oncol. 24, 179–185 (2013)
10. W. E. Mesker, J. M. C. Junggeburt, K. Szuhai, P. de Heer, H. Morreau, H. J. Tanke, and R. A. E. M. Tollenaar, The carcinoma-stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage. Cell. Oncol. 29, 387–98 (2007)
11. J. H. Park, C. H. Richards, D. C. McMillan, P. G. Horgan, and C. S. D. Roxburgh, The relationship between tumour stroma percentage, the tumour microenvironment and survival in patients with primary operable colorectal cancer. Ann. Oncol. 25, 644–651 (2014)
12. N. P. West, M. Dattani, P. McShane, G. Hutchins, J. Grabsch, W. Mueller, D. Treanor, P. Quirke, and H. Grabsch, The proportion of tumour cells is an independent predictor for survival in colorectal cancer patients. Br. J. Cancer. 102, 1519–1523 (2010)
13. G. W. van Pelt, T. F. Hansen, E. Bastiaannet, S. K. Frifeldt, J. H. J. van Krieken, R. A. E. M. Tollenaar, F. B. Sorensen, and W. E. Mesker, Stroma-high lymph node involvement predicts poor survival more accurately for patients with stage III colon cancer. J. Med. Surg. Pathol. 1, 1–8 (2016)
14. O. G. F. Geessink, A. Baidoshvili, G. Freling, J. M. Klaase, C. H. Slump, and F. van der Heijden, Toward automatic segmentation and quantification of tumor and stroma in whole-slide images of H&E stained rectal carcinomas. Proc. SPIE Medical Imaging: Digital Pathology. 9420, 0F1–0F7 (2015)
15. A. Wright, D. Magee, P. Quirke, and D. E. Treanor, Towards automatic patient selection for chemotherapy in colorectal cancer trials. Proc. SPIE Medical Imaging: Digital Pathology. 9041, 0A1–0A8 (2014)
16. F. Bianconi, A. Álvarez-Larrán, and A. Fernández, Discrimination between tumour epithelium and stroma via perception-based features. Neurocomputing. 154, 119–126 (2015)
17. N. Linder, J. Konsti, R. Turkki, E. Rahtu, M. Lundin, S. Nordling, C. Haglund, T. Ahonen, M. Pietikäinen, and J. Lundin, Identification of tumor epithelium and stroma in tissue microarrays using texture analysis. Diagn. Pathol. 7, 22 (2012)

18. B. Ehteshami Bejnordi, M. Veta, P. J. van Diest, B. van Ginneken, N. Karssemeijer, G. Litjens, J. A. W. M. van der Laak, M. Hermsen, Q. F. Manson, M. Balkenhol, O. G. F. Geessink, N. Stathonikos, M. C. R. F. van Dijk, P. Bult, F. Beca, A. H. Beck, D. Wang, A. Khosla, R. Gargeya, H. Irshad, A. Zhong, Q. Dou, Q. Li, H. Chen, H. J. Lin, P. A. Heng, C. Haß, E. Bruni, Q. Wong, U. Halici, M. Ü. Öner, R. Cetin-Atalay, M. Berseth, V. Khvatkov, A. Vylegzhanin, O. Kraus, M. Shaban, N. Rajpoot, R. Awan, K. Sirinukunwattana, T. Qaiser, Y. W. Tsang, D. Tellez, J. Annuscheit, P. Hufnagl, M. Valkonen, K. Kartasalo, L. Latonen, P. Ruusuvuori, K. Liimatainen, S. Albarqouni, B. Mungal, A. George, S. Demirci, N. Navab, S. Watanabe, S. Seno, Y. Takenaka, H. Matsuda, H. A. Phoulady, V. Kovalev, A. Kalinovsky, V. Liauchuk, G. Bueno, M. M. Fernandez-Carrobles, I. Serrano, O. Deniz, D. Racoceanu, and R. Venâncio, Diagnostic assessment of deep learning algorithms for detection of lymph node metastases in women with breast cancer. JAMA. 318, 2199–2210 (2017)
19. G. Litjens, C. I. Sánchez, N. Timofeeva, M. Hermsen, I. Nagtegaal, I. Kovacs, C. Hulsbergen-Van De Kaa, P. Bult, B. van Ginneken, and J. A. W. M. van der Laak, Deep learning as a tool for increased accuracy and efficiency of histopathological diagnosis. Sci. Rep. 6, 26286 (2016)
20. F. Ciompi, O. G. F. Geessink, B. E. Bejnordi, G. S. De Souza, A. Baidoshvili, G. J. S. Litjens, B. van Ginneken, I. D. Nagtegaal, and J. A. W. M. van der Laak, The importance of stain normalization in colorectal tissue classification with convolutional networks. Proc. IEEE Int. Symp. Biomed. Imaging. 14, 160–163 (2017)
21. QUASAR Collaborative Group, Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 370, 2020–2029 (2007)
22. R. Glynne-Jones, L. Wyrwicz, E. Tiret, G. Brown, C. Rödel, A. Cervantes, and D. Arnold, Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 28, iv22-iv40 (2017)



CHAPTER 7

General discussion

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GENERAL DISCUSSION

Going digital for diagnostics is one of the most important and challenging use cases of digital pathology. In the last eight years the team at LabPON (Laboratory for Pathology Eastern Netherlands) solved many issues in creating a workflow for digital diagnostics, invested a lot of time and money and did research. Now we can better address our remaining challenges and come up with solutions.

These were the steps that we needed to take for successful implementation of digital diagnostics as laid out in the General Introduction:

1. Make a workflow analysis of your laboratory.
2. Make a business plan and implementation plan.
3. Optimize the information technology (IT) infrastructure.
4. Select, purchase and validate a suitable scanner.
5. Adjust laboratory logistics.
6. Customize the workplace of pathologists.
7. Organize training and education of pathologists for digital diagnostics.
8. Integrate the IMS (Image Management System) into LMS (Laboratory Information Management System) and develop and Install distribution software.
9. Set up and safe use of an external network for consultation and remote work.
10. Develop, validate and use image analysis software.

We will now one by one discuss the progress made in these steps described in this thesis.

Make a workflow analysis of your laboratory

A realistic business case for the implementation of digital pathology can only be made on basis of a detailed flow analysis and a step-wise of all individual elements of this analysis. In Chapter 4 we modelled and analysed, for the 5 most common workflows, the differences between the traditional analogue and new digital ways of working, carried out detailed measurements of all relevant steps in key analogue and digital processes, and compared time spent. On average, leaving out the time individual pathologists needed for their evaluation of slides, in total over 19 hours (of which about 1 hour of pathologists' time) was saved on a typical day by working digitally, with the highest savings in the routine diagnosis and multi-disciplinary meeting workflows. Saving about 19 hours per day translates into 2.63 fte laboratory staff (36 hours working week), corresponding to about 120k euros per year.

Make a business plan and implementation plan

We argue that a business case should not only contain financial items but also better ergonomics, increased user satisfaction of pathology and clinical staff, higher attraction to young doctors for the residency program, increased speed of diagnosis (and thereby lower anxiety and indirectly also work dropout of patients and their relatives), and last but not least high patient safety

and increased quality of diagnosis, the latter saving significant costs outside the pathology laboratory as pointed out by Ho et al (1). The IMS as case viewer offers new possibilities, allowing the pathologists to see at a glance the diagnostic cases scheduled for that day. They can see the daily list of tissue numbers, section numbers and medical service requests, and sort this list by preference. The software keeps track of what the pathologist has seen per case study and per section.

In LabPON we also compared the time required to perform microscopic assessment by pathologists between a conventional workflow (using an optical microscope and glass slides) and digitized whole slide images in Chapter 5. The study was performed in a primary diagnostic setting and comprised >3800 cases in total. While earlier studies often found that a digital workflow required more time from the pathologists (2, 3), we found that for most case types the digital workflow required significantly less time by the pathologist. Overall, an average time gain of 12.3% was observed in favour of the digital workflow. It was concluded that transitioning from conventional microscopy to whole slide images significantly reduces the pathologists' reading times. Saving about 12.3% of pathologist time correspond to about 20k euros per year per pathologist (about 160k gross salary per year). In LabPON we have 19 pathologist and with thereby we can save about 380k per years, more than 2 fte.

In addition to the time savings found in this study in case reading, pathologists time may be gained in other tasks as well, such as preparation for tumor boards and consultations (1). We hope to assess this in further studies. Finally, it is to be expected that algorithms based on artificial intelligence will eventually significantly reduce pathologists' time, e.g. by prescreening lymph slides for presence of tumor (4). Altogether, the time savings on the lab staff and pathologist level may help to close the business case for going digital pathology, together with the increase in quality of diagnostics and patient safety.

Optimize the information technology (IT) infrastructure

In Chapter 2 we provide information about optimization of IT infrastructure. Several elements play an important part in the diagnostics workflow of digital pathology in order to ensure the working convenience and speed of work. One of them is the speed of image rendering within the viewer. Faster image rendering results in a quicker diagnosis. For rapid image rendering proper equipment such as a fast network, fast server and a good computer are indispensable.

Speed of network is important. In our laboratory we have a network with a minimum of 1 GB/s, while 400 Mb/s appeared to be good enough for digital diagnostics. Network speed between server and scanners needs to be much faster in view if vast data transfer and in LabPON this network part is 10 GB/s.

For calculation of required storage, the number of slides and the amount of tissue on the slides, as well as the desired storage duration are important features. We calculated that at LabPON we need 25TB of storage every month. We store our images for 8 weeks because most multidisciplinary meetings will occur within that time. At this moment, LabPON can unfortunately not afford to store all WSI permanently. We are waiting for new developments

that will make storage more cost-effectively, e.g. by compressing images, and consider to store images at 20x. This position is similar to that of other (non-academic) pathology laboratories in The Netherlands that diagnose partly with WSI. Some academic centers, such as the University Medical Center in Utrecht, store all their WSI permanently, but they archive their glass slides at the same time awaiting regulation that will allow to discard the slides.

Select, purchase and validate a suitable scanner

We did extensive market research, tested several scanners and eventually validated a scanner in our laboratory as described in Chapter 2. Here, it needs to be considered whether fluorescent and big slides will need to be scanned and if z-stack scanning is required for e.g. cytology, since not all scanner types provide these options. The initial validation of a scanner was done with 173 diagnostic cases comprising 1042 slides representing a normal day of this routine diagnostic laboratory, in compliance with CAP guidelines. These cases were digitally diagnosed by a panel of three pathologists. After three days, the panel diagnosed the exact same material through a light microscope. We found that WSI was good enough in comparison with light microscopic images, and we did not encounter disturbing technical problems.

After deciding on the scanner, you need to know how many scanners are required. The most accurate calculations are based on data from LMS. Of course, slides are not produced continuously, and this creates slide production peaks for which more scanning capacity is needed. Priority slides, IHC, cytology and other specifics also need to be considered. Based on a real life calculation we bought 5 scanners for parallel scanning to reduce the scanning time, while theoretical calculations suggested that only 2 should be sufficient.

Adjust laboratory logistics

LabPON has five high-throughput Ultra-Fast Scanners (UFS). The technicians usually run four scanners simultaneously. Most of the daily production of slides are scanned at night and distributed in the morning to the available pathologists. However, because of the continuous, nonstop workflow, the technicians also use the scanners during the day and divide the slides equally among the scanners. Racks are used simultaneously with a total capacity of 300 slides. Average scanning time in LabPON is +/- 2.5 minutes per slides, so +/- 50 minutes for one rack of 20 slides. During the peak times there is more than 400 MB of data transferred from the scanners to server each second, requiring sufficient network bandwidth.

Digitalisation changed the flow of the entire process in the laboratory. After digitalisation we adapted many of the protocols. Occasionally, mechanical problems occurred within the scanners when grabbing or moving slides during the scanning process. The scanner will then give an error message and will stop processing. This problem was often related to slide related issues, such as sticky, not well dried out, incorrectly covered or labelled slides. We adapted our coverslipper and we made our labels smaller and consequently the slides do not stick anymore. Presently, we have less than 5 errors per week with 5 scanners and most of our errors are solved by our technical services in 5 minutes.

Customize the workplace of pathologists

The work place of a pathologist must be ergonomically correct and allow to work quickly and efficiently. Pathologist must move images all day with a mouse, which may lead to RSI (repetitive strain injury). Using different devices helps, and together with Philips, we have developed several tools for different devices.

Philips made efforts to minimize the number of clicks by developing an efficient user interface which allows the use of keyboard shortcuts. This resulted in user-friendly software that is more intuitive to work with. Together with Philips we developed a touchpad tool and a tool for the "Space Navigator" to prevent RSI symptoms. The touchpad works in the same way as a tablet or mobile phone and allows pathologists to choose their user interfaces.

Scanner companies advised us as to which computers were necessary for digital diagnosis with minimum technical requirements and configurations. However, they were often not good enough. We found that computers with a faster processor work 2 times faster digitally than other computers (Chapter 1) and bought new ones. Other computer specifications may also play a part such as the graphics card, since digital pathology requires images of high quality and a minimum of least two monitors.

In LabPON, we have experimented with monitors and the outcome is that almost all standard high resolution monitors are good enough for diagnostics. We found that three things are important: Use a minimum of 2 monitors between 24 and 30 inches of the same size and the same models, because each monitor has different letter sizes and different colours, even after calibration.

Although this may be personal, the best size seems to be 27 inches. The screen should not be too small to see all the required details, but also not too big, otherwise the pathologist will risk too much head movements.

Integrate IMS into LMS and develop and install distribution software

At LabPON, we implemented comprehensive bidirectional integration with our Laboratory Information Management System (LIMS) to optimally benefit from the improvement in efficiency and quality that digital pathology solutions offer. Every day, material arrives from three hospitals and from regional general practitioners. After processing of the material and staining, the slides are dispatched to all pathologists present that day and a diagnosis is made and reported.

The Finalist LIMS was already used for a long time to handle specimens in the lab and to track the steps in processing from arrival and registration of a case up until sending of the final report to the requesting physician. To ensure efficient and secure data handling, an adjusted bidirectional workflow was essential. In practice, the use of barcodes ensures that tissue is traceable during the entire route. Every step in the process, from the entry of patient data and clinical information until signing out the final report, is logged.

The barcode generated by the Finalist LMS is read by the Philips Ultra Fast Scanner (UFS). The barcode information and the image data as yielded by the UFS are coupled by the Philips Image Management System (IMS) server. The IMS in turn exchanges information with the LIMS server. The

UFS and IMS are part of Philips IntelliSite Pathology Solution, an open platform for digital pathology. The information transferred to the IMS is shown both in the work list of the LMS and the IMS case viewer as well as on the individual slide images within the case. The work list contains all cases that an individual pathologist needs to review. The system will trigger the LMS to show the matching report of the cases open in IMS, preventing mix-ups while reporting. It is possible to start with opening a case viewer and reporting from either the LIMS or the IMS. Relevant patient information is shared between the LIMS and the IMS viewer. Cases handled by IMS are checked and evaluated by LIMS and vice versa. The full and bidirectional integration of both systems warrants accurate matching of cases. Any change in one system will lead to appropriate matching in the other.

The experience at LabPON clearly shows that communication between LIMS and IMS, when applied fully and in a bidirectional way, makes it possible to access images as well as process information and reports while eliminating the risk of mixing up cases.

Set up and safe use external network including consultation and remote work

We tested the feasibility of a digital teleconsultation network for remote consultation in pathology using WSI in Chapter 3. Three laboratories participated with in total 90 scanned cases. Each laboratory was asked to review the cases from the two other labs and to score relevant technical issues. The case mix was relatively complex, including renal, bone marrow and lymph node biopsies with malignant lymphomas, as well as a few sarcoma specimens, allowing evaluation of the network for consultation purposes. Importantly, all 16 pathologists were familiar with digital pathology but, with the exception of a few, were not daily practising it, precluding any positive prejudice to digital pathology.

The digital network indeed appeared to be suitable for consultation purposes with only a few technical issues limiting its use. In particular, the speed of case retrieval and building images were not optimal, nevertheless, not perceived as a major nuisance by the 16 involved pathologists. Some slides showed parts that were out of focus as well, however, focus quality was generally felt to be sufficient. In only one case with a very small focus of prostatic adenocarcinoma out-of-focus problems hindered the diagnostic process.

The re-diagnoses were completely concordant with the original diagnosis in 89% of the cases. When eight minor discrepancies were not taken into account the concordance rate rose to 88/90, i.e. 98%. Moreover, reanalysis of the discordances showed that only two (minor and major) discrepancies could be attributed to the use of digital pathology (2%), while the other eight discrepancies were most likely due to differences in awareness and interpretation by the individual pathologists.

This high overall concordance rate was within the range of previously observed inter- and intra-observer variability in digital and microscopic pathology in general and is in line with previous single institute studies by our group (5-8) and others (9-14). Together, these results indicate that consultation using WSI in an inter-laboratory network is feasible and sufficiently reliable. Latency time scores were not optimal, obviously leaving room for improvement.

In the Netherlands a new initiative, project PIE (Pathology Image Exchange), started three years ago to realize a national platform for exchange of WSI between Dutch pathology labs for consultation, revision and pathology panels. This project is in the meanwhile operational and allows to process cases for digital revision and consultation with all the relevant patient data available in a secure environment.

Develop, validate and use image analysis software

Digital pathology has caused a revival of image analysis in pathology, especially by deep learning approaches, and many algorithms will soon be available. As an example, we describe in Chapter 6 an algorithm for assessing tumor-stroma ration in colorectal cancer. Tumour-stroma ratio has been shown to yield prognostic information in various cancer types (15). To the best of our knowledge, our work shows for the first time that TSR assessed by an automatic deep learning algorithm is a strong independent prognostic factor both for DSS and DFS in rectal carcinoma patients. Remarkably, we showed the prognostic value of TSR-auto in stage II patients, both in terms of DSS and DFS, which is a relevant result in the context of personalized post-operative treatment of rectal carcinoma patients, where the definition of an optimal strategy for TNM stage II patients is still under debate.

The deep learning based approach proposed in this work needs the manually provided position of a hot-spot as input to assess TSR. As such, the intervention of an expert is still required, making the method only semi-automatic. It is worth noting that in an other study (16) the computer model used in this work has shown high performance at segmenting several tissue types in rectal cancer at whole-slide image level, i.e., beyond the limited area of the selected hot-spot. As a consequence, this method has the potential to be used to quantify TSR both at whole-tumour level and at whole-slide image level. Such an approach would overcome the need for a user-provided hot-spot and therefore allow to investigate TSR at very large scale via fully-automatic computation.

The objectiveness of a deep learning based method, which allows to obtain accurate and reproducible quantification of TSR, has the potential to address the problem of subjectivity and lack of reproducibility of currently used visual approaches to TSR estimation, contributing to pave the way to the implementation of TSR in clinical practice.

Overall conclusion

Much progress has been made over recent years in creating workflows for fully digital diagnostics, and the different studies in this thesis have contributed to that. We expect digital a digital diagnostic pathology revolution within the next 5 years.

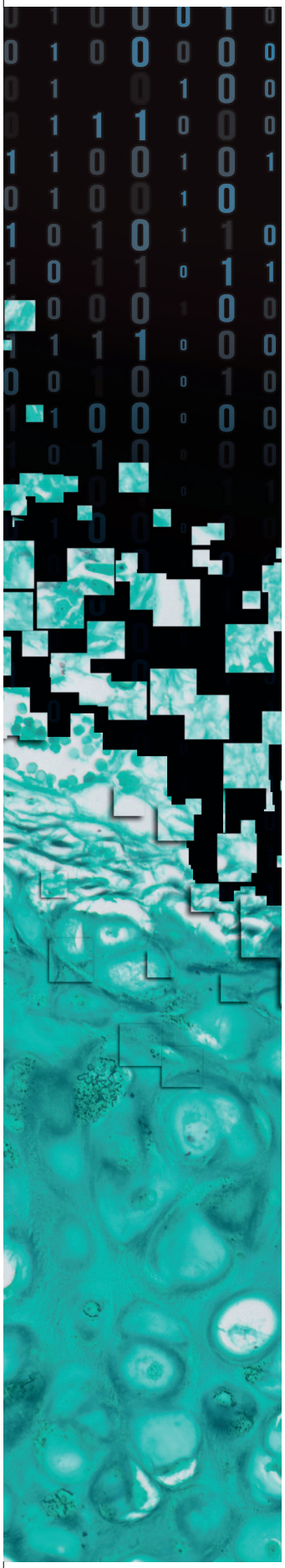
REFERENTIES

1. Ho J, Ahlers SM, Stratman C, et al. Can digital pathology result in cost savings? A financial projection for digital pathology implementation at a large integrated health care organization. J Pathol Inform. 2014 Aug 28; 5:33. doi: 10.4103/2153-3539.139714. eCollection 2014.
2. Treanor D, Quirke P. The Virtual Slide and Conventional Microscope - a direct comparison of their diagnostic efficiency. *J Pathol*. 2007; 213: 7a.
3. Velez N, Jukic D, Ho J. Evaluation of 2 whole-slide imaging applications in dermatopathology. *Hum Pathol*. 2008; 39: 1341--1349.
4. Ehteshami Bejnordi B, Veta M, van Diest PJ, et al. Diagnostic assessment of deep learning algorithms for detection of lymph node metastases in women with breast cancer. *JAMA*. 2017; 318: 2199-2210.
5. Al Janabi S, Huisman A, Vink A, et al. Whole slide images for primary diagnostics in dermatopathology: a feasibility study. J Clin Pathol 2012; 65:152-158.
6. Al Janabi S, Huisman A, Vink A, et al. Whole slide images for primary diagnostics of gastrointestinal tract pathology: a feasibility study. Hum Pathol 2012; 43:702-707.
7. Al Janabi S, Huisman A, Nap M, Clarijs R, van Diest PJ. Whole slide images as a platform for initial diagnostics in histopathology in a medium sized routine laboratory. J Clin Pathol 2012; 65:1107 1111.
8. Al Janabi S, Huisman A, Willems SM, Van Diest PJ. Digital slide images for primary diagnostics in breast pathology: a feasibility study. Hum Path 2012; 43:2318 2325.
9. Goacher E, Randell R, Williams B, Treanor D. The Diagnostic Concordance of Whole Slide Imaging and Light Microscopy: A Systematic Review. Arch Pathol Lab Med. 2017; 141:151-161.
10. BJ Williams et al; A systematic analysis of discordant diagnoses in digital pathology compared with light microscopy. Arch Pathol Lab Med 2017; 141:1712-1718.
11. Furness P. A randomized controlled trial of the diagnostic accuracy of internet-based telepathology compared with conventional microscopy. Histopathology 2007; 50:266-273.
12. Wilbur DC, Madi K, Colvin RB, et al; Whole-slide imaging digital pathology as a platform for teleconsultation: a pilot study using paired subspecialist correlations. Arch Pathol Lab Med 2009; 133:1949-1953.
13. Jones NC, Nazarian RM, Duncan LM, et al; Interinstitutional whole slide imaging teleconsultation service development: assessment using internal training and clinical consultation cases. Arch Pathol Lab Med 2015; 139:627-35.
14. Bauer TW, Slaw RJ; Validating whole-slide imaging for consultation diagnoses in surgical pathology. Arch Pathol Lab Med 2014; 138:1459-1465.
15. ScheerRoeke et al., 2017Scheer, R., Baidoshvili, A., Zoidze, S., Elferink, M. A. G., Berkel, A. E. M., Klaase, J. M., van Diest, P. J. (2017). Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: A retrospective cohort study. *World Journal of Gastrointestinal Oncology*, 9(12), 466-474.
16. Ciompi et al., 2017Ciompi, F., Geessink, O., Ehteshami Bejnordi, B., de Souza, G. S., Baidoshvili, A., Litjens, G., van Ginneken, B., Nagtegaal, I., and van der Laak, J. (2017). The importance of stain normalization in colorectal tissue classification with convolutional networks. In *Proc. IEEE 14th Int Symp Biomed Imaging*, pages 160–163.

PART 3

SUMMARY

Summary in English
Summary in Dutch
Summary in Georgian



Summary

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SUMMARY

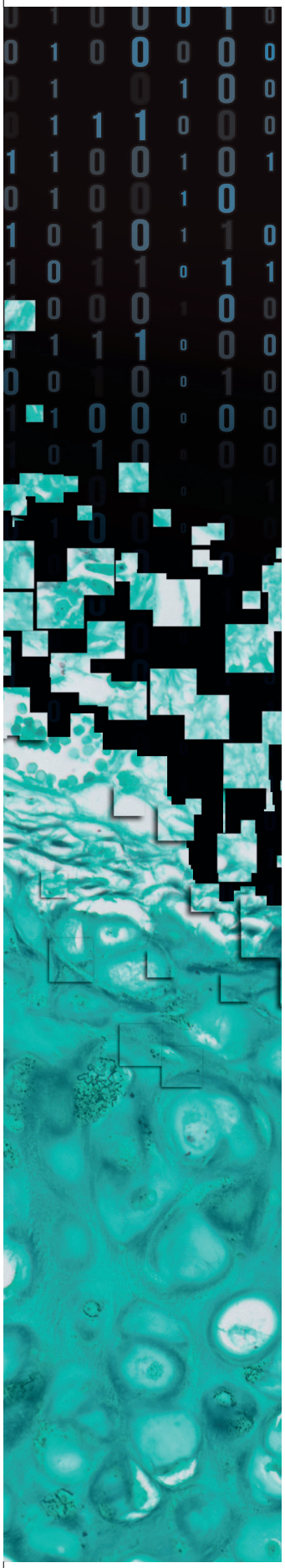
In pathology laboratories human tissues are cut into thin slices that are placed on glass slides which in turn are stained and covered with a thin layer of glass. These glass slides are then examined under the microscope by a pathologist. For centuries this workflow has essentially stayed the same while in other medical areas the transition to digital techniques has brought many changes. For instance, in radiology the transition has recently been made from X-ray films to digital images.

This has not yet been done in pathology despite the possibility to digitize microscopic images. For a while now digital images and videos have been made from tissues samples and cells and more recently it is become possible to make digital images with a whole slide scanner. This provides the pathologist with the possibility to view microscopic images on a computer screen. It is pivotal that the scanned microscopic images are of good quality and resemble the images seen through the microscope as much as possible. Therefore, it is important to investigate whether diagnosing digitally does not have any negative effects and does not result in delay, but instead perhaps has benefits for the pathologist and patient. It is expected that there would be improvement in quality, safety and efficiency. When LabPON started digitizing microscopic images, few other pathology laboratories had relevant experience in this field. LabPON in liaison with other partners started testing several scanners and digital pathology software to improve the workflow for digital diagnostics. This thesis describes this process.

After the transition to digital diagnostics LabPON put a lot of effort into optimizing the working conditions for pathologists. It is well known that there are health issues related to working with the microscope. In principle, these ergonomic complaints can be prevented when working digitally for diagnosing tissues. On the other hand, new health issues (like repetitive strain injury) may arise for the pathologist. At LabPON we tested different types of computers and monitors as well as new user interphases were used and adapted to create an optimal work environment for the pathologist, as described in chapter 2.

In order to work efficiently for the pathologist the Laboratory Information and Management System (LIMS) and Image Management System (IMS) are completely integrated into the LabPON workflow. Permutations of patient information and material can be prevented by working in this manner. Another interesting feature of working with the digital method of diagnosing is that it is possible to work outside of LabPON and enables you to easily consult specialists from other pathology laboratories. In chapter 3 we compared the quality of consultation with tissue slides and the digital method of consulting tissues. The digital consultation was more efficient than sending patient material (i.e. tissue slides) by post and the time for diagnosing a patient was reduced by 1 to several days. In addition, the risk of damaging or losing unique patient material when sending by post is eliminated. In chapter 4 we demonstrate that with the transition to digital diagnostics we saved 19 hours of lab and administrative work per day in LabPON, i.e. more than 2 fte. This makes digital diagnostics more efficient and more cost effective. With diagnosing digitally the pathologist seems to be 12% more efficient than with the microscope. This is described in chapter 5.

With the use of special software, digital tissue images can be analysed as well. This software can improve the efficiency but also the quality of the diagnosis. An additional benefit of using software is that it can do a lot of pre-work for the pathologist. De advances of using software in digital pathology is described in chapter 6. At LabPON together with our partners we demonstrated that the tumor cell and tumor stroma ratio in colon cancer can be determined more accurately when using digital pathology software. In general, digitalization of diagnostics in pathology laboratories and the use of image analysis software will help improve the quality and efficiency in pathology practice, as well as improving patient care.



Samenvatting

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SAMENVATTING

In pathologie laboratoria worden zachte kleurloze menselijke weefsels verwerkt tot dunne plakjes op glaasjes die gekleurd en daarna afgedekt worden om onder de microscoop te bekijken. Er is al eeuwen weinig aan dit proces veranderd. Bij de radiologie is recent een overstap gemaakt van beelden op röntgenfilms naar digitale beelden op beeldschermen.

Bij de pathologie is dit nog niet gebeurd ondanks dat er al jaren vele middelen bestaan om microscopische beelden te digitaliseren. Al lang worden digitale video's en foto's van weefsel en cellen gemaakt en sinds kort kunnen ook van complete weefselcoupes met een scanner digitale beelden worden gemaakt. Dit geeft de mogelijkheid om microscopische beelden van het weefsel op een beeldscherm te bekijken in plaats van onder de microscoop. Het is daarbij belangrijk om te zorgen dat gescande beelden zo goed mogelijk lijken op datgene dat onder de microscoop wordt gezien. Hiervoor is het belangrijk om te onderzoeken of de digitale methode van diagnostiek geen nadelen heeft voor de patiënt. Er mogen geen risico's voor patiënten zijn en er mag geen vertraging in de diagnostiek optreden. Anderzijds is het belangrijk de potentiele voordelen van de digitale beoordeling goed in kaart te brengen waarbij ernaar wordt gestreefd dat er een verbetering in kwaliteit, veiligheid en efficiëntie is.

Toen we op LabPON met digitalisering begonnen waren er geen laboratoria met relevante ervaring in digitalisering van routine diagnostiek. LabPON heeft samen met zijn partners onderzoek verricht, en apparaten en software uitgetest en verbeterd om te komen tot een optimale workflow voor digitale diagnostiek. Over dit proces gaat dit proefschrift.

In LabPON hebben we ook veel gedaan om na de overgang naar digitale diagnostiek de werkomstandigheden voor de patholoog te optimaliseren om te zorgen dat de patholoog veilig en ergonomisch digitaal kan werken. Het is bekend dat sommige pathologen gezondheidsklachten hebben door het werken met de microscoop. Zulke ergonomische klachten kunnen in principe voorkomen worden bij de overstap naar digitale diagnostiek, terwijl nieuwe soorten gezondheidsklachten moeten worden voorkomen. Daarom hebben we in LabPON computers en monitors getest, nieuwe user interfases (zoals alternatieven voor de klassieke "muis") in gebruik genomen en aangepast voor het werken met digitale beelden om een optimale en efficiëntie werkplek voor de patholoog te creëren. Dit kunt u vinden in hoofdstuk 2.

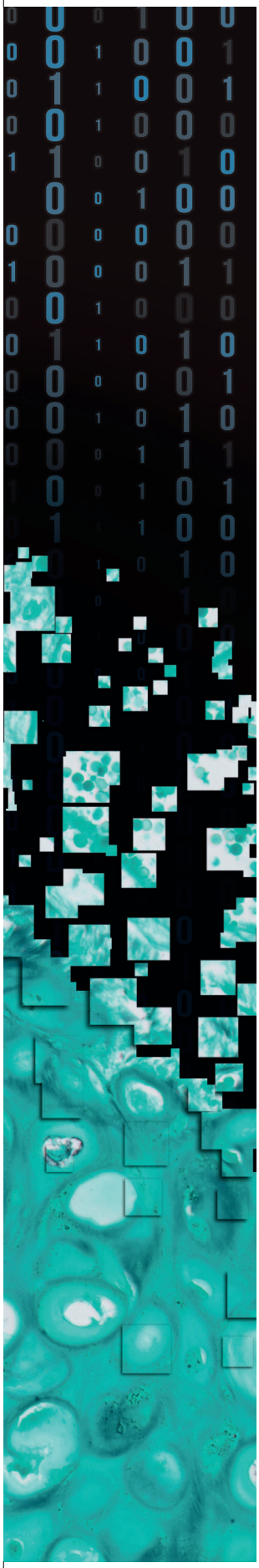
In LabPON hebben we ons Laboratorium Informatie en Management Systeem en Image Management Systeem compleet geïntegreerd om een veilig werkproces voor de patholoog te creëren, verwisseling van patiënten materiaal te voorkomen en efficiëntie te verhogen.

Digitalisering geeft ons ook de mogelijkheid om op afstand buiten LabPON te werken. Ook het consulteren van superspecialisten buiten LabPON wordt makkelijker. In hoofdstuk 3 hebben we onderzocht of digitale consulten op afstand kwalitatief dezelfde zijn als consulten met glazen weefselcoupes die per post worden opgestuurd. Digitale consulten kunnen veel sneller gedaan worden dan de conventionele, en dit vermindert de diagnostiek tijd voor de patiënt van ongeveer twee weken naar één tot enkele dagen. Daarnaast is het risico van beschadiging of verlies van uniek materiaal bij verzending per post verdwenen.

Het door ons in LabPON verricht onderzoek heeft aangetoond dat de overgang van de conventionele naar digitale diagnostiek het laboratorium veel logistieke voordelen oplevert. In hoofdstuk 4 hebben we aangetoond dat we in LabPON bij overgang naar digitale diagnostiek maar liefst 19 uur werk van analisten en secretariaat per dag besparen. Dit maakt digitale diagnostiek efficiënter en goedkoper. Ook de diagnostiek door de patholoog blijkt veel sneller te gaan: in hoofdstuk 5 laten we zien dat bij de overgang naar digitale diagnostiek de ervaren "digitale patholoog" ruim 12% efficiënter werkt dan met een microscoop.

Met gebruik van software kunnen digitale beelden ook door de computer geanalyseerd worden. Hierbij wordt gebruik gemaakt van geheel nieuwe technieken, soms wel aangeduid met de term "deep learning". Deze software kan helpen in de verbetering van efficiëntie en kwaliteit van de diagnostiek. Door gebruik van dergelijke software kan de diagnostiek die momenteel subjectief en patholoog afhankelijk is meer objectief gemaakt worden, hetgeen zal resulteren in een kwalitatieve vooruitgang van onze diagnostiek. Met dergelijke software kan ook automatisch veel voorwerk (screening) voor de patholoog worden verricht zodat de patholoog veel efficiënter kan werken. Voordelen van gebruik van dergelijke software in de digitale pathologie laten we zien in hoofdstuk 6. Daar hebben we samen met onze partners aangetoond dat met behulp van nieuwe software nauwkeuriger kwantitatieve analyses verricht kunnen worden van de verhouding tussen tumorcellen en bindweefsel in dikke darmkanker.

Digitalisatie van diagnostiek in pathologie laboratoria en gebruik van beeldanalyse software kan helpen met verbetering van kwaliteit en efficiëntie van diagnostiek in pathologie laboratoria. Het zal een goede bijdrage leveren voor verbetering van patiëntenzorg.



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მოკლე შინაარსი

ჰისტოპათოლოგიურ ლაბორატორიაში, სპეციალური დამუშავების შემდეგ, რბილი და შეუფერავი ქსოვილოვანი ფრაგმენტებისაგან მიიღება თხელი ანათალი, რომელიც განთავსთება მინაზე. შემდგომში პრეპარატი იღებება, იფარება საფარი მინით და ხდება მისი მიკროსკოპული გამოკვლევა. საუკუნეების განმავლობაში ამ პროცესმა ძალიან მცირე სახეცვლილება განიცადა, მაშინ, როდესაც თანამედროვე ციფრულმა მეთოდებმა მედიცინის სხვა მიმართულებებში უკვე მნიშვნელოვნი გარდაქმნები შეიტანა, მაგალითად, არც თუ ისე დიდი ხნის წინ, რადიოლოგია რენტგენის ფირებიდან სრულად გადავიდა ციფრული გამოსახულებების გამოყენებაზე. დიგიტალიზაცია არ შეხებია პათოლოგიას, მიუხედავად იმისა, რომ უკვე მრავალი წელია არსებობს მიკროსკოპული პრეპარატების ციფრულ ფორმატში გადაყვანის მრავალი საშუალება, მაგალითად, ქსოვილის ცალკეული ფრაგმენტისა თუ უჯრედების ციფრული ფოტო- თუ ვიდეოგადაღების სახით. არც თუ ისე დიდი ხნის წინ კი ასევე შესაძლებელი გახდა მინაზე მოთავსებული ქსოვილის სკანირება და მისი სრულად კომპიუტერის ეკრანზე, მიკროსკოპის გარეშე დათვალიერება. თუმცა ამ შემთხვევაში მნიშვნელოვანია, რომ სკანირების შედეგად მიღებული სურათის ხარისხი არ ჩამოუვარდებოდეს მიკროსკოპული გამოსახულების ხარისხს. აქედან გამომდინარე, მნიშვნელოვანია, გამოკლვლეულ იქნეს დიაგნოსტიკის ციფრულ მეთოდის უარყოფითი მხარეები, რამაც შესაძლოა გავლენა იქონიოს პაციენტზე - მაგალითად, ისეთ ფაქტორებზე, როგორიცაა საბოლოო დიაგნოზის სიზუსტე, ასევე დიაგნოზის გაცემის დრო. მეორე მხრივ, მნიშვნელოვანია ციფრული მეთოდის პოტენციური უპირატესობების ნათლად გამოვლენაც, რამაც შესაძლოა, გააუმჯობესოს დიაგნოსტიკის ხარისხი, პაციენტის უსაფრთხოება და გაზარდოს სამუშაო პროცესის ეფექტურობა.

იმ დროისთვის, როდესაც LabPON-ში პირველად დავიწყეთ დიაგნოსტიკის პროცესის დიგიტალიზაციაზე მუშაობა, არ არსებობდა სხვა ლაბორატორია, რომელსაც უკვე ჰქონდა მსგავსი გამოცდილება. იმისათვის, რომ ციფრული სამუშაო პროცესის ოპტიმიზაცია მოგვეხდინა, LabPON-მა პარტნიორებთან ერთად ჩაატარა სამეცნიერო კვლევები, გამოცადა და გააუმჯობესა სხვადასხვა აპარატურა და კომპიუტერული პროგრამა. სწორედ ამ პროცესების შესახებ მოგვითხრობს ეს სამეცნიერო ნაშრომი.

ციფრულ დიაგნოსტიკაზე გადასვლის შემდეგ LabPON - მა დიდი ძალისხმევა გასწია პათოლოგანატომებისთვის სამუშაო პირობების ოპტიმიზაციის მიზნითაც. საყოველთაოდ ცნობილია, რომ პათოლოგანატომების გარკვეულ ნაწილს აღენიშნება ჯანმრთელობასთან დაკავშირებული პრობლემები, რაც განპირობებულია მიკროსკოპთან ხანგრძლივი მუშაობით. ერთი მხრივ, მსგავსი ერგონომიული პრობლემები შეიძლება თავიდან იქნას აცილებული ციფრულ დიაგნოსტიკაზე გადასვლის შემდეგ, მაგრამ მეორე მხრივ, ასევე შესაძლოა თავი იჩინოს სხვა ტიპის ჩივილებმა. სწორედ ამ მიზეზების გამო LabPON-ში პრაქტიკაში

გამოვცადეთ სხვადასხვა ტიპის კომპიუტერები და მონიტორები, ასევე გამოვიყენეთ მომხმარებლის ახალი ინტერფეისები (მაგალითად სტანდარტული კომპიუტერის „თაგვის" თანამედროვე ალტერნატივები) და მოვარგეთ პათოლოგანატომების ახალ, დიგიტალურ სამუშაო გარემოს. ამ პროცესების შესახებ ინფორმაცია შეგიძლიათ იხილოთ მე-2 თავში.

იმისათვის, რომ გაგვეზარდა სამუშაო პროცესის ეფექტურობა და თავიდან აგვეცილებინა სხვადასხვა პაციენტის მასალის ერთმანეთში არევა, ჩვენ მოვახდინეთ ლაბორატორიის ინფორმაციის და მართვის სისტემისა (Laboratory Information Management System - LIMS) და გამოსახულების მართვის სისტემის (Image Management System – IMS) ინტეგრაცია.

ჰისტოპათოლოგიური ლაბორატორიის სამუშაო პროცესის ციფრულ მეთოდზე გადასვლა ასევე გვაძლევს საშუალებას ვიმუშაოთ დისტანციურად და გავამარტივოთ სუპერ სპეციალისტებთან კონსულტაციები (იქნება ეს ლაბორატორიის, რეგიონისა თუ ქვეყნის ფარგლებს გარეთ). მე-3 თავში ჩვენ ვაჩვენეთ, რომ დიგიტალური კონსულტაციები არათუ ხარისხით არ ჩამოუვარდება აქამდე გამოყენებულ მეთოდს (მიკროსკოპული პრეპარატების გზით, რომელთა გადაგზავნა ხდება ფოსტით), არამედ ამცირებს კონსულტაციის მიღების და პაციენტის საბოლოო დიაგნოზის გაცემის დროს რამდენიმე კვირიდან რამდენიმე დღემდე. ასევე პრაქტიკულად არ არსებობს ტრანსპორტირებისას მასალის დაკარგვის ან დაზიანების რისკი.

LabPON-ში ჩვენ მიერ ჩატარებულმა კვლევამ ასევე აჩვენა, რომ მუშაობის ტრადიციული მეთოდიდან ციფრულ მეთოდზე გადასვლას მოაქვს მრავალი უპირატესობა ლოგისტიკის თვალსაზრისითაც. მე-4 თავში ასახულია, თუ როგორ მოხერხდა ჩვენთან ამ გზით ლაბორანტებისა და მდივნების სამუშაო დროის მინიმუმ 19 საათით შემცირება დღეში განმავლობაში. ამგვარად ვაჩვენეთ, რომ ციფრული დიაგნოსტიკა არის ბევრად უფრო ეფექტური და იაფი. როგორც კვლევამ აჩვენა, ეს მეთოდი ამცირებს პათოლოგანატომის მიერ დიაგნოზის დასმის დროსაც: მე-5 თავში აღწერილია, თუ როგორ მუშაობს გამოცდილი „ციფრული პათოლოგანატომი" 12%-ით უფრო ეფექტურად ვიდრე მიკროსკოპის გამოყენებით მუშაობის დროს.

კომპიუტერული პროგრამის საშუალებით შესაძლებელია ციფრული გამოსახულების კომპიუტერული ანალიზიც. ამ შემთხვევაში გამოყენებულია ისეთი ინოვაციური ტექნოლოგიები, როგორიცაა, მაგალითად, ბოლო დროს სულ უფროდაუფრო ხშირად გამოყენებული „ღრმა დასწავლის მეთოდი" („deep learning"). ამგვარ კომპიუტერულ პროგრამას შეუძლია დაგვეხმაროს მუშაობის ხარისხისა და ეფექტურობის გაუმჯობესებაში. მსგავსი პროგრამების გამოყენებით შესაძლებელია დიაგნოსტიკა, რომელიც ამჟამად ძალიან სუბიექტურია და დამოკიდებულია კონკრეტულ პათოლოგანატომზე, გახდეს უფრო ობიექტური, რაც იქნება ხარისხობრივად წინ გადადგმული ნაბიჯი ჩვენს საქმიანობაში. მაგალითად, ასეთ კომპიუტერულ პროგრამას შეეძლება ავტომატურად გააკეთოს შემთხვევების წინასწარი დამუშავება (სკრინინგი), რაც მისცემს პათოლოგანატომს საშუალებას,

უფრო ეფექტურად შეასრულოს თავისი სამუშაო. მსგავსი კომპიუტერული პროგრამის გამოყენების უპირატესობები განხილულია მე-6 თავში. ჩვენ, ჩვენს პარტნიორებთან ერთად, ვაჩვენეთ, თუ ხარისხობრივად რამდენად უფრო ზუსტად არის შესაძლებელი სიმსივნური უჯრედებისა და შემაერთებელი ქსოვილის შეფარდების გამოთვლა მხვილი ნაწლავის კარცინომის შემთხვევაში ახალი კომპიუტერული პროგრამის საშუალებით.

ზოგადად, პათოლოგანატომიურ ლაბორატორიაში დიაგნოსტიკის დიგიტალიზაცია და კომპიუტერული პროგრამის საშუალებით ციფრული გამოსახულების ანალიზი ხელს შეუწყობს პათოლოგანატომიური სამსახურის ეფექტურობისა და ხარისხის, შესაბამისად ჯანდაცვის ხარისხის გაუმჯობესებას

PART 4

APPENDIX

Acknowledgements
Curriculum Vitae
List of Publications

Acknowledgements

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ACKNOWLEDGEMENTS

As a medical student I started working in research at the VU University Medical Centre Amsterdam under supervision of Prof. dr. J.W.M. (Hans) Niessen in the research group of cardiopathology. I am Hans Niessen grateful for his guidance throughout those years as well as for his medical expertise.

After my internships in 2005 I was given the opportunity to start my training as a pathologist at the VUMC. This was exactly what I was looking for in medicine. Combining my residency with my research was tough at times but due to the support of my pathology colleagues and resident physicians I was able to finish my pathology training in 2009. My gratitude goes towards all of them, but especially to former Head of Pathology Prof. dr. C.J.L.M. (Chris) Meijer for his support during resident at the VUMC.

In 2009 I applied for a position as a pathologist at LabPON in Enschede and with success. Here I started as a young pathologist. Former director of LabPON Henk van der Veen was supportive of innovation at LabPON and gave me the opportunity to start new projects among which the digitalization of pathology. Also I feel very thankful towards my colleagues for their support to start digitizing LabPON. As one of the first pathology laboratories we were able to completely digitize our workflow. This could not have happened with the tremendous effort of all our colleagues at LabPON and with our partner Philips. During the digitalization of our workflow I was also able to start up new research at LabPON. Since January 2018 we worked together with Philips on a new project called "Computational Pathology" that I am very pleased to lead. Due to the hard work of our team, Shorena Zoidze, Mariska Stellaard, Ana Japaridze - van Harsselaar, Maayke Boll and pathologists we are able to successfully push this project forward. I am grateful for their enthusiasm and competence. Shorena and Maayke have helped me tremendously at the end of my PhD. Thank you for being my paranymphs.

A special gratitude goes out towards Prof. dr. P. (Paul) van Diest. He is the reason that I have chosen the subject digital pathology for my PhD thesis. He came up with the idea and also accompanied me throughout the whole project. Prof. dr. P. Kluin and Dr. J.A.W.M. van der Laak have my gratitude for supporting me during my PhD work.

My work as a pathologist at LabPON, my travels abroad for congresses and my activities in Georgia have taken a lot of time, consequently being away from my family and friends. I am most grateful to my family, my mother and especially my children for being so understanding and supporting me along the way.

The unconditional and full support from my wife has made all this possible and I offer her my deepest gratitude.

Alexi Baidoshvili
12 September 2018, Enschede

Curriculum Vitae

524759-L-bw-Alexi
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CURRICULUM VITAE

Alexi Baidoshvili was born in Tbilisi, Georgia on the 21th of March 1968. After graduating from secondary school in Tbilisi in 1985 he went to military service. After three years of serving the military he was accepted as a medical student at the Tbilisi State Medical University. After coming to The Netherlands, he continued his medical studies at the Vrije Universiteit Amsterdam (VUMC). In 2002 he started working as a medical researcher at the department of Pathology at the VU Medical Centre Amsterdam. After obtaining his medical degree in 2005 he started his pathology residency at the VU Medical Centre Amsterdam. He received his board certification in Pathology in November 2009. He then started as a pathologist at Laboratorium Pathologie Oost-Nederland (LabPON) in 2009. In 2015 he started his PhD research at the Department of Pathology at the University Medical Centre Utrecht (UMCU).

List of publications

LIST OF PUBLICATIONS

Baidoshvili A, Bucur A, van Leeuwen J, van der Laak J, Kluin P, van Diest PJ. Evaluating the benefits of digital pathology implementation: time savings in laboratory logistics. Histopathology 2018 Jun 20. doi: 10.1111/his.13691.

Baidoshvili A, Stathonikos N, Freling G, Bart J, 't Hart N, van der Laak J, Doff J, van der Vegt B, Kluin PM, van Diest PJ. Validation of a whole-slide image-based teleconsultation network. Histopathology 2018 Jun 12. doi: 10.1111/his.13673.

Litjens G, Bandi P, Ehteshami Bejnordi B, Geessink O, Balkenhol M, Bult P, Halilovic A, Hermsen M, van de Loo R, Vogels R, Manson QF, Stathonikos N, **Baidoshvili A**, van Diest P, Wauters C, van Dijk M, van der Laak J. 1399 H&E-stained sentinel lymph node sections of breast cancer patients: the CAMELYON dataset. Gigascience 2018 Jun 1;7(6). doi:10.1093/gigascience/giy065. PMID: 29860392

Ciompi F, Geessink O, Ehteshami Bejnordi B, Silva de Souza G, **Baidoshvili A**, Litjens G, van Ginneken B, Nagtegaal I, van der Laak J. The importance of stain normalization in colorectal tissue classification with convolutional networks. EEE International Symposium on Biomedical Imaging, 2017 Apr, DOI: 10.1109/ISBI.2017.7950492), pages 160-163.

Scheer R, **Baidoshvili A**, Zoidze S, Elferink MAG, Berkel AEM, Klaase JM, van Diest PJ. Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: A retrospective cohort study. World J Gastrointest Oncol 2017 Dec 15;9(12):466-474. doi: 10.4251/wjgo.v9.i12.466. PMID: 29290917

Alibegashvili T, Clifford GM, Vaccarella S, **Baidoshvili A**, Gogiashvili L, Tsagareli Z, Kureli I, Snijders PJ, Heideman DA, van Kemenade FJ, Meijer CJ, Kordzaia D, Franceschi S. Human papillomavirus infection in women with and without cervical cancer in Tbilisi, Georgia. Cancer Epidemiol 2011 Oct;35(5):465-70. doi: 10.1016/j.canep.2010.12.006. Epub 2011 Feb 2. PMID: 21292583

Kupreishvili K, **Baidoshvili A**, ter Weeme M, Huybregts MA, Krijnen PA, Van Hinsbergh VW, Stooker W, Eijsman L, Niessen HW. Degeneration and atherosclerosis inducing increased deposition of type IIA secretory phospholipase A2, C-reactive protein and complement in aortic valves cause neutrophilic granulocyte influx. J Heart Valve Dis 2011 Jan; 20(1):29-36. PMID: 21404895

Baidoshvili A, Krijnen PA, Kupreishvili K, Ciurana C, Bleeker W, Nijmeijer R, Visser CA, Visser FC, Meijer CJ, Stooker W, Eijsman L, van Hinsbergh VW, Hack CE, Niessen HWM, Schalkwijk CG. N(varepsilon)-(carboxymethyl)lysine depositions in intramyocardial blood vessels in human and rat acute myocardial infarction: a predictor or reflection of infarction? Arterioscler Thromb Vasc Biol 2006 Nov;26(11):2497-503. Epub 2006 Sep 14. PMID: 16973974

Baidoshvili A, Niessen HW, Stooker W, Huybregts RA, Hack CE, Rauwerda JA, Meijer CJ, Eijsman L, van Hinsbergh VW, Schalkwijk CG. N(omega)-(carboxymethyl)lysine depositions in human aortic heart valves: similarities with atherosclerotic blood vessels. Atherosclerosis. 2004 Jun;174(2):287-92. PMID:15136058

Schalkwijk CG, **Baidoshvili A**, Stehouwer CDA, van Hinsbergh VWM, Niessen HWM. Increased accumulation of the glycoxidation product Nepsilon-(carboxymethyl)lysine in hearts of diabetic patients: generation and characterisation of a monoclonal anti-CML antibody. Biochim Biophys Acta 2004 Mar 22;1636(2-3):82-9. PMID: 15164755

Stooker W, Gök M, Sipkema P, Niessen HW, **Baidoshvili A**, Westerhof N, Jansen EK, Wildevuur CR, Eijsman L. Pressure-diameter relationship in the human greater saphenous vein. Ann Thorac Surg 2003 Nov; 76(5):1533-8. PMID: 14602282

Dodge-Khatami A, Niessen HWM, **Baidoshvili A**, van Gulik TM, Klein MG, Eijsman L, de Mol BAJM. Topical vascular endothelial growth factor in rabbit tracheal surgery: comparative effect on healing using various reconstruction materials and intraluminal stents. Eur J Cardio-Thorac Surgery 2003 Jan;23(1):6-14. PMID:12493496

Baidoshvili A, Nijmeijer R, Lagrand WK, Hack CE, Niessen HWM. Localization of C-reactive protein in infarcted tissue sites of multiple organs during sepsis. J Clin Pathol 2002 Feb;55(2):152-3. PMID: 11865015

Nijmeijer R, Lagrand WK, **Baidoshvili A**, Lubbers TP, Hermens Wth, Meijer CJLM, Visser CA, Hack CE, Niessen HWM. Secretory type II phospholipase A(2) binds to ischemic myocardium during myocardial infarction in humans. Cardiovasc Res 2002 Jan;53(1):138-46. PMID: 11744022

Stooker W, Niessen HWM, **Baidoshvili A**, Wildevuur WR, van Hinsbergh VWM, Fritz J, Wildevuur CRH, Eijsman L. Perivenous support reduces early changes in human vein grafts. J Thorac Cardiovasc Surg 2001 Aug;122(2):233. PMID: 11174734