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Faculty of Medicine
Doctoral Programme in Clinical Research
University of Helsinki

POINT-OF-CARE DIAGNOSTICS WITH DIGITAL MICROSCOPY AND ARTIFICIAL INTELLIGENCE

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ACADEMIC DISSERTATION

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“När tänkte du bli docent?”

- Monika Carpelan-Holmström, mamma, 2019

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I. **Holmström O.**, Linder N., Lundin M., Moilanen H., Suutala A., Turkki R., Joensuu H., Isola J., Diwan V., Lundin J. Quantification of estrogen receptor-alpha expression in human breast carcinomas with a miniaturized, low-cost digital microscope: a comparison with a high-end whole slide-scanner. *PLoS ONE 10.12 (2015): e0144688.*

II. **Holmström O.,** Linder N., Ståhls A., Nordling S., Moilanen H., Suutala A., Lundin M., Linder E., Diwan V., Lundin J. Detection of breast cancer lymph node metastases in frozen sections with a point-of-care low-cost microscope scanner *PLoS ONE 14.3 (2019): e0208366.*

III. **Holmström O.,** Linder N., Kaingu H., Mbuuko N., Mbete J., Kinyua F., Törnquist S., Muinde M., Krogerus L., Lundin M., Diwan V., Lundin J. Screening for cervical cancer by artificial-intelligence analysis of Pap smears at a peripheral clinic in Kenya. *Manuscript, 2020*

IV. **Holmström O.**, Linder N., Ngasala B., Mårtensson A., Linder E., Lundin M., Moilanen H., Suutala A., Diwan V., Lundin J. Point-of-care mobile digital microscopy and deep learning for the detection of soil-transmitted helminths and Schistosoma haematobium. *Global Health Action 10 Sup. 3 (2017): 1337325.*

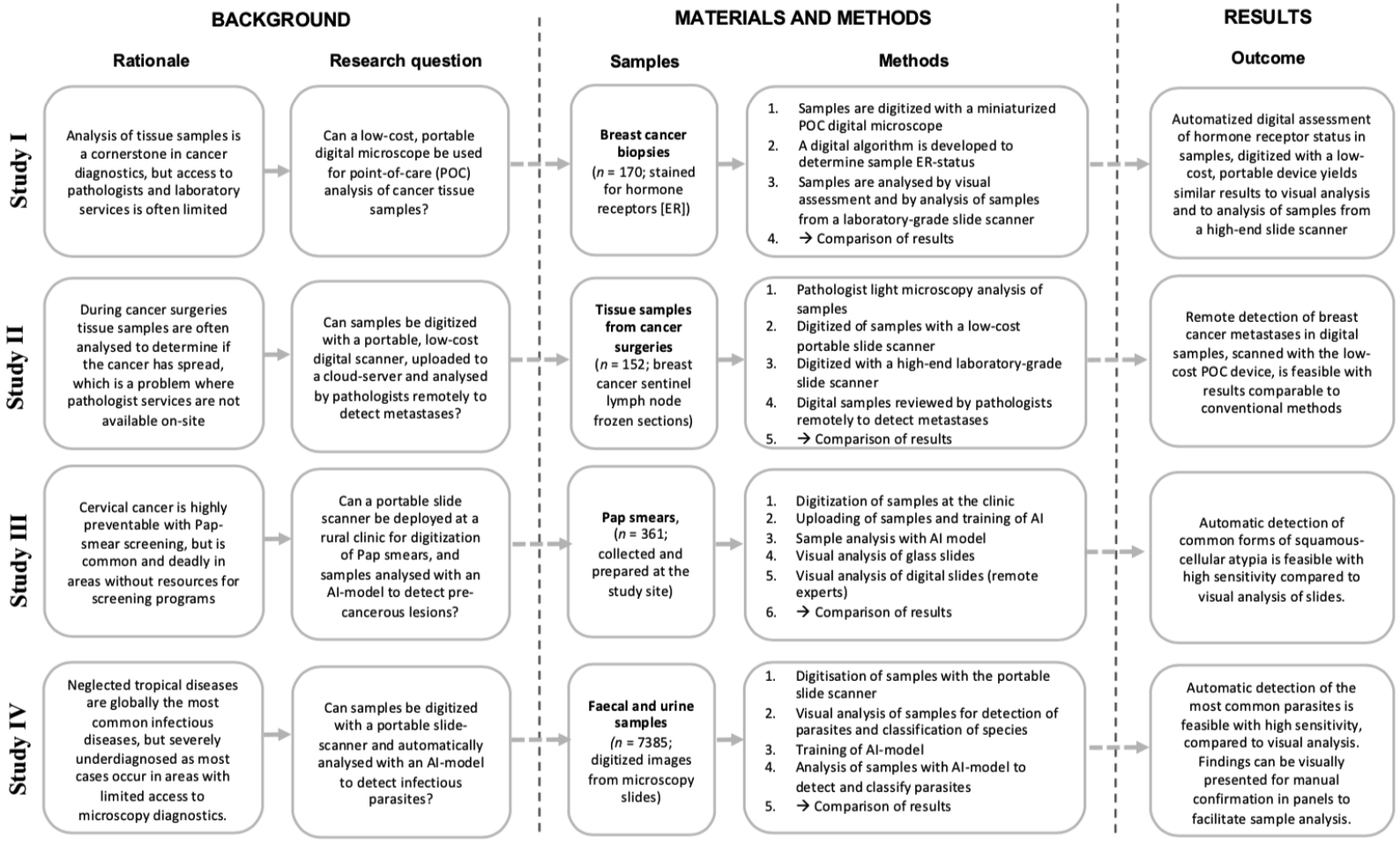
The publications are referred to in the text by their roman numerals. The original publications are reprinted with the permission of their copyright holders.

ABBREVIATIONS

κ	kappa-value
95% CI	95% confidence interval
AI	artificial intelligence
ANN	artificial neural network
ALND	axillary lymph node dissection
ASC-H	atypical squamous cells, cannot exclude HSIL
ASC-US	atypical squamous cells of undetermined significance
AUC	area under the curve
CAD	computer-aided diagnosis
CIN	cervical intraepithelial lesion
CMOS	complementary metal oxide semiconductor
CNN	convolutional neural network
CV	computer vision
DAB	diaminobenzidine
DLS	deep learning system
ECW	enhanced compressed wavelet
ER	estrogen receptor
FFPE	formalin-fixed paraffin embedded
FIMM	Institute for Molecular Medicine Finland
FOV	field of view
FN	false negative
FP	false positive
FS	frozen section
GT	ground truth
H&E	haematoxylin and eosin
HDR	high dynamic range
HSIL	high-grade squamous intraepithelial lesion
HPV	human papillomavirus
IHC	immunohistochemistry
ITC	isolated tumour cell(s)
JPEG	Joint Photographic Experts Group (compression format)
LBC	liquid-based cytology
LED	light-emitting diode
LSIL	low-grade squamous intraepithelial lesion
ML	Machine learning
NA	numerical aperture
NPV	negative predictive value
NTD	neglected tropical diseases
PBS	phosphate buffer solution
PNG	portable network graphics

POC	point-of-care
PPV	positive predictive value
PR	progesterone receptor
RGB	red, green and blue colour system
ROC	receiver operating characteristic
SCC	squamous cell carcinoma
SSL	secure socket layer
TN	true negative
TP	true positive
TEMED	tetraacetylethylenediamine
TIFF	tagged image file format
TMA	tissue microarray
UV	ultra violet
VGA	video graphics array
WSI	whole-slide image

Brief overview of Thesis



ABSTRACT

The lack of access to diagnostics is a global problem which causes underdiagnosis of various common and treatable diseases. In certain areas, the access to laboratory services and medical experts is extremely limited, such as in sub-Saharan Africa, with often less than one practising pathologist per one million inhabitants. Annually, hundreds of millions of microscopy samples are analysed to diagnose e.g. infectious diseases and cancers, but the need for more is significant. During the last decade, technological advancements and reduced prices of optical components have enabled the construction of inexpensive, portable devices for digitization of microscopy samples; a procedure traditionally limited to well-equipped laboratories with expensive high-end equipment. By allowing digitization of samples directly at the point of care (POC), advanced digital diagnostic techniques, such as the analysis of samples with medical 'artificial intelligence' (AI) algorithms, can be utilized also outside high-end laboratories – which is precisely where the need for improved diagnostics is often most significant.

The aim of this thesis is to study how low-cost, POC digital microscopy, supported by automatized digital image analysis and AI can be applied for routine microscopy diagnostics with an emphasis on potential areas of application in low-resource settings.

We describe, implement and evaluate various techniques for POC digitization and analysis of samples using both visual methods and digital algorithms. Specifically, we evaluate the technologies for the analysis of breast cancer tissue samples (assessment of hormone receptor expression), intraoperative samples from cancer surgeries (detection of metastases in lymph node frozen sections), cytological samples (digital Pap smear screening) and parasitological samples (diagnostics of neglected tropical diseases).

Our results show how the digitization of a variety of routine microscopy samples is feasible using systems suitable POC usage with sufficient image quality for diagnostic applications. Furthermore, the findings demonstrate how digital methods, based on computer vision and AI, can be utilized to facilitate the sample analysis process to e.g. quantify tissue stains and detect atypical cells and infectious pathogens in the samples with levels of accuracy comparable to conventional methods.

In conclusion, our findings show how technological advancements can be leveraged to create general-purpose digital microscopy diagnostic platforms, which are implementable and feasible to use for diagnostic purposes at the POC. This allows the utilization of modern digital algorithms and AI to aid in analysis of samples and facilitate the diagnostic process by automatically extracting information from the digital samples. These findings are important steps in the effort to develop novel diagnostic technologies which are usable also in areas without access to high-end laboratories, and the technologies described here are also likely to be applicable for diagnostics of other diseases which are currently diagnosed with light microscopy. To our knowledge, no similar academic project of this magnitude that investigates these diagnostic technologies has been conducted.

1 INTRODUCTION

Light microscopy has for centuries remained the relatively unchanged diagnostic standard for a number of diseases (Farahani, Monteith, 2016). The examination of samples with light microscopes is widely utilized in clinical work and scientific research, although having certain downsides - such as being labour-intensive, time-consuming and prone to subjectivity. One practical limitation of the technique is the requirements for trained experts on-site to perform the sample analysis process, which is a problem in areas lacking access to medical experts. Digital microscopy and computer-aided diagnostics have been proposed as solutions, but the shift towards digital methods in the field of microscopy diagnostics has been slow. The introduction of slide scanners during the end of the 20th century enabled microscopy samples to be digitized for remote viewing and analysis using digital methods, and technological advancements since has enabled large-scale digitization of samples at high resolution (Pantanowitz et al., 2018). Digital technologies can potentially facilitate the analysis of samples in a number of ways by e.g. allowing remote viewing of samples, automatically detecting objects of interest, improving turnaround time for analysis of slides and increase diagnostic accuracy, but the adaptation has been slow especially in routine clinical work (Montalto, 2016). One limitation to wider-scale adaptation of digital microscopy is the requirement for expensive and bulky digitization equipment, which has limited the techniques mainly to well-equipped laboratories (Isaacs et al., 2011, Hernández-Neuta et al., 2019). During recent years, technologies have been developed to enable digitization of microscopy samples also outside the laboratory, using devices which are significantly smaller and more cost-efficient compared to traditional alternatives. The potential for this type of devices to provide platforms for digital diagnostics in field settings, by utilizing e.g. camera phone components, has been recognized in multiple studies (Boppart, Richards-Kortum, 2014; Vasiman, A., Stothard, J.R. & Bogoch, I.I. 2019). In parallel, the field of digital image analysis is undergoing a paradigm shift with the rise of ‘artificial intelligence’ (AI) for medical diagnostics. These novel software algorithms have demonstrated a remarkable performance in complex image-analysis tasks, such as the analysis of microscopy samples (Bera et al., 2019), and could provide important diagnostic support tools and reduce the workload for clinicians especially in resource-constrained areas.

Lacking access to laboratory services and diagnostics negatively impacts the treatment of diseases and outcome for the patient (Adesina et al., 2013) and the global need for improved front line diagnostic testing is significant in many geographical regions for multiple diseases (World Health Organization, 2017). By leveraging these digital diagnostic techniques, there is a potential to improve access to diagnostics and healthcare on a global level (Figure 1).

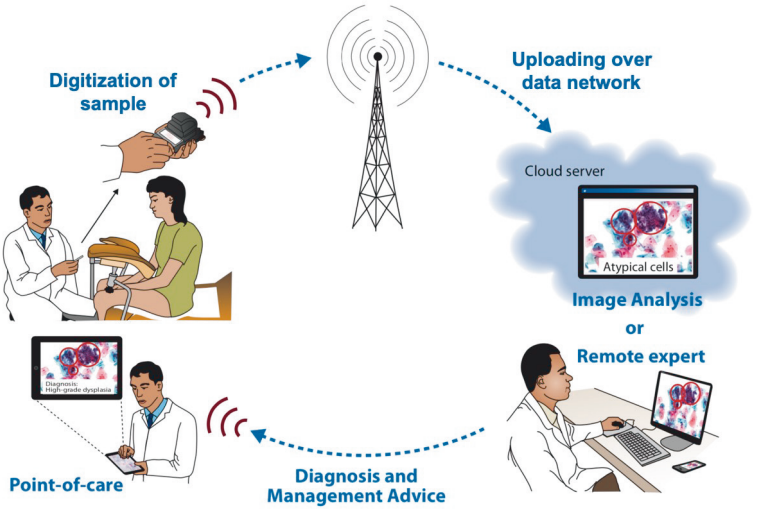


Figure 1. Point-of-care digital diagnostic workflow. Schematic image illustrating steps in a digital diagnostic pipeline which includes sample acquisition, digitization, uploading, analysis and verification of results.

To study the diagnostic potential of the technologies here, we describe, develop and evaluate several approaches to digital microscopy supported by automatized digital image analysis methods, which are feasible for POC usage. We apply these methods for diagnostics of various common conditions, which would be likely to benefit significantly from improved front line diagnostics. Specifically, we study the analysis of cancer tissue samples for assessment of hormone receptor status (ER-receptors in breast cancer), detection of metastases in lymph node frozen sections, the cytological screening for cervical cancer and the diagnostics of the most common neglected tropical diseases (NTDs).

2 REVIEW OF THE LITERATURE

2.1 From optical to digital microscopy

Optical microscopy, or light microscopy, is the traditional type of microscopy that utilizes visible light and an arrangement of one or more lenses to magnify images of microscopy samples. The light microscope is one of the oldest instruments still in use in medical diagnostics and scientific research, and has remained relatively unchanged since the 17th century (Chen, Zheng & Liu, 2011). Optical microscopy has been the diagnostic standard for a variety of diseases by allowing visual analysis of specimens at high magnification to enable visualization of pathogens, such as bacteria and parasites and assessment of detailed tissue morphology and structure - features which are vital e.g. in cancer histopathology (Chan, 2014). The resolving power of a conventional light microscope is mainly determined by the objective lens used and is dependent on the wavelength of the light source used. For conventional optical microscopes (using visible light) this means that the resolving power is theoretically limited to approximately 0.2 µm, due to the wavelength of visible light (~400 nm). Although conventional microscopes remain essential tools for clinicians, the visual microscopy examination of samples has certain downsides, such as being time-consuming, labour-intense, prone to subjectivity and requiring the presence of trained microscopists on-site. During the last decades, there has been an increased interest in the field of digital microscopy and digital pathology (Pantatonowitz et al., 2011). Digital microscopy is a field which combines pathology with digital technologies (Griffin, Treanor, 2017). Digital imaging systems, such as digital microscopes or whole-slide scanners, are platforms capable of transforming physical microscopy specimens (glass slides) into digital formats and represents a key component in the digital pathology workflow. Modern high-resolution slide scanners support digitization of specimens at sub-micrometre resolution into digital images, so-called whole-slide images (WSIs) (Figure 2). Similarly to optical microscopes, the specimen in digital microscopes is also magnified with an objective lens, but the corresponding image is relayed directly (or through lens arrays) to an electronic detector (i.e. light-sensitive sensor, such as a CCD or CMOS sensors), which samples the continuous (analogue) signal into discretely-sized data in the form of pixels in the digital image. The image sensor ideally has a pixel pitch (density of pixels) smaller than the minimum resolvable distance by the objective lens to avoid degradation of the resolving power by the

sensor. Thus, in digital systems the resolving power, or spatial resolution, is determined not only by the objective lens used but also the features of the sensor, e.g. the number of pixels and distance between pixels (the so-called sampling interval), and also the features of the display monitor (Sellaro et al., 2013). The sampling frequency required to accurately reproduce features in the digital image is determined by the Nyquist criterion, and is approximately equal to twice the highest spatial frequency of the specimen. This implies that, for example, at the diffraction limit of 0.2 µm for visible light, a digitizer must be capable of sampling at intervals that correspond to 0.1 µm or less to accurately capture details in the sample. While the optical magnification of conventional light microscopes is most often expressed with a dimensionless coefficient (e.g. 5x, 10x or 100x) which describes the ratio between the image observed through the microscope and the size of the object as viewed with the naked eye, digital magnification is measured differently. As the digital image consists of discrete pixels captured by the image sensor, the pixels represent the absolute upper limit of the image's resolution (i.e. no visual information exists which is "smaller" than the pixels). This image is viewed on a display with a separate resolution, which in turn determines the part of the image which is reproduced (e.g. for one sensor pixel to equal one pixel of the display, a standard 1920 x 1080-pixel monitor can only display a small part of a 40 000 x 40 000-pixel digital slide at a given time). When adjusting the digital magnification (zooming) in the digital image, the ratio between the sensor pixels and the monitor pixels is changed, but the information in the image file is not altered. As the size of the display can vary (e.g. a cell phone or computer screen), it is necessary to know the physical size of the individual pixels to be able to determine the ratio between the size of the object on the sample and the displayed image on the monitor. Given the dimensions of microscopic features in pathology, this per-pixel spatial metric is often expressed as µm/px for digital microscopes to allow unambiguous comparison of images from different systems. High-end whole slide scanners typically support digitization of slides at high magnification using 20x or 40x objectives with pixel sizes of 0.50 – 0.25µm/px (Evans et al., 2017). Although the imaging performance of digital microscopes typically is described only with the magnification power of the objective lens and the sensor pixel size, the actual resolving power (minimum resolvable distance) of the system depends on the combination of components used, but typically is around 2-2.5 times the pixel size for high-end scanners which corresponds to approximately ~0.5 µm (Zarella et al., 2018). Whole-slide scanners utilize digital microscope systems with robotics to automatically move the

glass slides while simultaneously focusing and capturing image data from different locations in the sample. The corresponding WSI is constructed by assembling individual images, consisting of square-shaped separate fields-of-views (FOVs) from the sensor (tile-based scanners), or longer image strips (line-based scanners), into a single image file containing the whole digitized sample, in a process called stitching. Digitized, i.e. "scanned", samples are stored either on local hard drives or uploaded to cloud servers for remote access before viewing. *Virtual microscopy* is a broad term used for viewing and sharing of digital samples, e.g. for applications in remote diagnostics or education. The term *telepathology* is commonly used specifically to refer to the practice of pathology at a distance by remote viewing of WSIs. The utilization of digital microscopy allows digital image analysis methods to be used to aid in sample analysis. For this, the term *computer-aided diagnosis* (CAD) has been used to describe digital pathology (and other image-based medical fields) with digital image analysis. Currently, conventional whole slide digitization systems are mainly limited to well-established and advanced laboratories, as the implementation of these systems is expensive and require extensive supporting digital infrastructure (e.g. network, storing, viewing of slides), dedicated trained personnel to operate the equipment and regular service and maintenance (Isaacs et al., 2011, Zarella et al., 2018).

Figure 2. Whole slide scanner at FIMM laboratory (University of Helsinki). Image showing high-end whole-slide scanner **(1)**, which is connected to a desktop computer **(2)** used to control the device and manage the digitized samples (whole slide images).

2.1.1 Structure of a digital microscopy workflow

A typical digital pathology (microscopy) workflow consists of certain fundamental stages; **digitization** of samples, **interface** for accessing and viewing digital samples, **sharing** of data, data **archiving** and **digital analysis** (Pantanowitz et al., 2011).

Conventionally, the *digitization* of samples is performed with whole-slide scanners, as discussed in the previous section. Compared to conventional examination of samples with light microscopes, the *interface* for viewing digital WSIs is typically a computer display. Although being fundamentally different, this method has certain advantages, such as allowing the viewing of larger sample areas at a given time, improved ergonomics and potentially faster turnaround time for sample analysis (Vodovnik, 2016). By transferring physical samples into digital format, *sharing* and remote access to digital samples is possible over the internet, by e.g. uploading of slides to shared servers (Williams, Bottoms & Treanor, 2017). This provides opportunities for collaboration and has been widely implemented in areas such as education, research and remote consultations (Pantanowitz et al., 2018). Compared to the storage of physical samples, *archiving* and retrieving of digital samples is more convenient than storing and retrieving collections of glass slides. Notably, as the size of high-resolution WSIs can be substantial (multiple gigabytes), requirements in terms of IT infrastructure and hard drive capacity can be considerable for more sizeable collections of slides. Finally, the digitization of samples has resulted in significant new opportunities for *digital analysis* of samples and CAD. By utilizing automatized digital algorithms and software the analysis of samples can be facilitated by e.g. automatically detecting objects of interest, classification of samples and quantification of tissues and histological stains (Bera et al., 2019). This is discussed in more detail in the corresponding section on computer-aided microscopy diagnostics (2.3).

2.2 Digital microscopy at the point of care

Access to routine, real-time images of microscopy samples in all resource settings could significantly contribute to improving screening, diagnosis and treatment monitoring of various common diseases (Boppart, Richards-Kortum, 2014). POC diagnostics is an alternative to laboratory-based testing, where diagnostic information is provided in an outpatient setting to aid in clinical decision-making and reduce the time and equipment

required. During the last decade, a number of portable image-based solutions for POC diagnostic testing have been developed and tested for medical applications (Zhu et al., 2013; Saeed, Jabbar, 2017; Vasiman, A., Stothard, J.R. & Bogoch, I.I. 2019). Compared to conventional imaging solutions, such as traditional slide-scanners, this type of platforms offers multiple potential advantages, such as being more cost-efficient and portable, while still enabling real-time imaging of biological samples near the patient for early detection of diseases (Taruttis, Ntziachristos, 2012). By utilizing mobile phone connectivity and global data networks, captured images can be instantaneously shared remotely for expert consultations or uploaded to cloud servers e.g. for image analysis purposes (Smith et al., 2011). Inadequate or delayed access to diagnostics negatively affects the outcome for the patient, leads to additional visits and unnecessary referrals and delays initiation of adequate treatment interventions (Adesina et al., 2013). By leveraging advances in telecommunication technology and optics, there is an opportunity to develop novel solutions for improved diagnostics at a global level (Boppart, Richards-Kortum, 2014). In addition to providing a platform for rapid, front-line digital microscopy in low-resource areas without access to laboratory infrastructure, POC digital microscopy has potential applications also in higher-resource areas to facilitate analysis of samples e.g. in locations without on-site pathologists; such as operating suites where assessment of samples is required to guide the surgeon during operations, e.g. by determining tumor margins and detecting metastatic tissue (Pleijhuis et al., 2009).

2.2.1 Important features of point-of-care diagnostic platforms

As traditional digital microscopy and whole slide-scanning solutions tend to be high in cost and large in size, they are not ideal for POC applications in rural settings. To address these limitations, efforts have been made to develop compact and cost-effective solutions. According to the World Health Organization (WHO), POC diagnostic tests and equipment should meet the so-called ASSURED criteria by being Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users (Peeling, R. W. et al., 2006). Especially in low-resource environments, the minimization of cost is particularly important for both the device itself and per-test cost of sample analysis (Drain et al., 2014). Ideally, POC imaging devices should be small in size to allow for portability and durable

enough to withstand environmental factors, such as extreme weather conditions, presence of water and moisture and resistant to dust and disinfection protocols (Yager, Domingo & Gerdes, 2008). Especially in peripherally located and resource-constrained areas, the lack of infrastructure often means that continuous, stable access to power is limited, favouring solutions where alternate power sources, such as batteries, can be used. As access to trained medical and technical personnel in rural areas is limited, devices should preferably be usable with minimal training while still providing rapid, reliable results to aid in clinical decision making without the need for follow-up visits or additional patient contacts. Furthermore, possible local supply chain limitations need to be considered when developing digital microscopy solutions for low-resource areas (Boppart, Richards-Kortum, 2014).

2.2.2 Miniaturized digital microscopes as platforms for medical diagnostics

To date, several cost-efficient, miniaturized solutions for digitization of biological samples have been developed and tested for field diagnostics of a variety of diseases (Perkel et al, 2017). Many of these devices have been tested in laboratory conditions (Saeed, Jabbar, 2017), but several have also been evaluated in clinical settings; mainly in Africa for diagnostics of parasitic diseases (Vasiman, A., Stothard, J.R. & Bogoch, I.I. 2019) (Table 1). In general, for most current POC digital imaging systems, the highest achievable spatial resolution is lower (~1 µm) compared to the maximum spatial resolution achievable for typical laboratory high-end whole slide scanners (~0.5 µm). However, as a number of difference technological approaches have been studied for these applications, the resolving power of the systems varies substantially depending on the components used (e.g. 0.63 – 2.5 µm) (Zhu et al., 2013), and generalisation is therefore difficult.

The majority of the work on bringing optical imaging for medical diagnostics to the POC has been conducted during the last decade. Even before this, early studies such as the one conducted by Stothard et al. in 2005, demonstrated how infectious parasites (*S. mansoni*) could be visualized in field-settings in samples with an inexpensive, handheld field-microscope (with an attached single lens reflex camera to capture images) with reasonably high levels of sensitivity (85%) and specificity (96%) (Stothard et al., 2005). Following this, innovations and improvements in engineering, optics and digital technologies have resulted in significantly more advanced devices, which have shown

potential for field diagnostics of a variety of diseases. At an early stage, the potential to leverage smartphone technologies to create small-sized digital imaging devices was recognized. In 2009, Breslauer et al. demonstrated in a proof-of-concept study how mounting a camera phone to a microscope enabled visualization of malaria parasites (*P. falciparum*; in blood smears), tuberculosis bacilli (in Auramine-o stained sputum smears) and atypical erythrocytes for sickle-cell anaemia diagnostics (in blood smears), thus demonstrating that the imaging performance achievable with a smartphone is sufficient to resolve features essential for diagnostic purposes (Breslauer et al., 2009). During this year, the field of portable microscopy was also significantly advanced by an initiative from the Wellcome Trust, which resulted in the development of the Newton NM1 microscope. This commercially available device uses LED technologies in a compact portable microscope to enable field microscopy with similar levels of magnifications as laboratory microscopes, and also allows capturing of digital images by the attachment of a camera phone to the device. This device remains one of the most studied POC microscopes and has been used to achieve high levels of sensitivity and specificity (> 90%) for field diagnostics of malaria and the soil-transmitted helminths *A. lumbricoides, T. trichiura* and *S. mansoni* (Bogoch et al 2014; Coulibaly et al 2016). Apart from brightfield microscopy, fluorescence microscopy was recognized at an early stage to be usable to improve diagnostic performance e.g. for diagnostics of tuberculosis bacilli and malaria parasites, while still utilizing inexpensive components. Multiple field studies have reported results for this application that are comparable to laboratory-grade traditional microscopy (Albert et al., 2010). Field diagnostics of malaria with fluorescence POC microscopy has been studied with the battery-powered field fluorescence microscope 'Cyscope'; representing a portable, battery-powered fluorescent microscope. This device has been used to visualize malaria parasites in blood smears (Hassan et al., 2011) and for tuberculosis diagnostics (Chang, E., Page, A. & Bonnet, M. 2016), with similar levels of sensitivity and specificity (~90%) in multiple studies, although notably one study has reported high rates of false positives with this device for unclear reasons (Sousa-Figueiredo et al., 2010).

In 2013, Bogoch et al. showed how a simplified field digital microscope could be constructed by simply attaching a ball lens directly to a smartphone camera, and used it to diagnose schistosomiasis and various STHs in field settings (Bogoch et al., 2013 and 2014). Although the achieved sensitivity was modest in these studies, likely due to the limited FOV and spatial image quality, the device was simple and inexpensive; suggesting the

technology could be suitable for wider-scaler implement for diagnostics in field settings. To improve the image quality and FOV of smartphone camera systems, Switz et al. constructed a field-microscopy device for diagnostics of the same parasites which instead of a ball-lens utilized a second, reversed camera lens attached to a smartphone camera (Switz, D'Ambrosio & Fletcher, 2014) to produce a larger FOV (~10mm2) and a higher spatial resolution. Another device which utilizes a similar setup is the reverse-lens CellScope, developed by Fletcher et al. This device is a 3D-printed external module which is attached to the back of a camera phone to allow imaging of microscopy samples, e.g. for the diagnosis of *Schistosoma* and intestinal protozoa with high specificity (but only moderate sensitivity) (Ephraim et al., 2015; Coulibaly et al., 2016). A modified version of this device, called the LoaScope, has shown promise in the analysis of peripheral blood samples for the detection of the *Loa loa* parasite with high levels of reported sensitivity and specificity (100% and 94%) (D' Ambrosio et al., 2015). Similarly, high specificity (100%) but moderate (72%) sensitivity was also reported for diagnostics of schistosomiasis in a field study in Ghana by Bogoch et al., using another 3D-printed optoelectronic microscopy assembly that was attached to a smartphone (Bogoch et al., 2017). Apart from devices that utilize smart phone camera systems and miniaturized traditional compound microscope assemblies, a number of innovative approaches to digitizing samples at the POC have been described during the last decade. In 2014, a prototype of an extremely low-cost microscope (the 'Foldscope'), assembled from folded pieces of cardboard with a battery-powered LED light-source and a small-sized optical lens was presented (Cybulski, Clements & Prakash, 2014). Although this device can be manufactured extremely cost-efficiently (less than one USD), the usability is limited by the design (sample and device need to be examined in close proximity to the face of the user which presents a hygiene issue), and low degrees of sensitivity and specificity (Ephraim et al., 2015). Another approach to developing simple and compact POC optical imaging devices is lens-free holographic microscopy, described by Mudanyali et al. for detection of *Giardia lamblia* parasites (Mudanyali et al., 2010). After this, the technique has been applied to imaging of other sample types, such as cytological samples and blood smears (Greenbaum et al., 2012; Coskun, Ozcan, 2014). In addition to these imaging modalities, other original methods for medical imaging of microscopy samples have been described, relying on e.g. tomography, fluorescence, high-resolution wide-field lenses and movement analysis of schistosome miracidia (Zhu et al., 2013, Linder, Varjo & Thors, 2016). Although a number of these early-stage creative approaches have promising implications

for POC digital microscopy diagnostics, development is still needed before clinical evaluation is feasible.

Table 1. Summary of previous field studies investigating handheld point-of-care microscopes as diagnostic platforms with diagnostic characteristics of devices studies. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) shown with associated 95% confidence intervals (95% CI) when reported.

Study, author	Study location	Number of samples, *n*	Disease	Sensitivity, % (95%CI)	Specificity, % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)	Technology and/or device used
Stothard et al. 2005	Uganda	685	Schistosomiasis					
			S. mansoni	85.0	96.0	95.0	88.0	Meade Readview
Sousa-Figueiredo et al., 2010	Uganda	1530	Malaria					
			P. falciparum (adults)	86.7 (79.3-92.2)	38.8 (33.6-44.1)	32.8 (27.7-38.3)	89.4 (83.4-93.8)	Cyscope
			P. falciparum (children)	92.1 (89.6-94.1)	28.6 (22.8-34.9)	77.1 (73.9-80.2)	57.9 (48.3-67.1)	Cyscope
Hassan et al., 2010	Sudan	293	Malaria					
			P. falciparum (adults)	98.2 (90.6-100)	98.3 (95.7-99.5)	93.3 (83.8-98.2)	99.6 (97.6-100)	Cyscope
Hassan et al., 2011	Sudan	128	Malaria					
			P. falciparum (pregnant women)	97.6 (92.2-99.6)	89.1 (77.5-95.9)	94.1 (87.4-97.8)	95.3 (85.4-99.2)	Cyscope
Nkruma et al., 2011	Ghana	263	Malaria					
			P. falciparum (children)	100 (96.6-100)	97.4 (93.6-99.3)	96.4 (91-99)	100 (97.6-100)	Cyscope
Bogoch et al. 2013	Tanzania	199	STHs					
			STHs (all infections)	69.4 (61.8-76)	61.5 (40.7-79.1)	92.3 (85.9-96)	23.2 (14.2-35.2)	Smartphone add-on
			A. lumbricoides	81 (65.4-90.9)	87.3 (80.7-91.9)	63 (48.7-75.4)	94.5 (89.1-97.4)	Smartphone add-on
			T. trichura	54.4(46.3-62.3)	63.4 (46.9-77.4)	85.1 (76.4-91.2)	26.5 (18.4-36.6)	Smartphone add-on
			Hookworm	14.3 (8.3-23.1)	89.1 (81-94.2)	56 (35.3-75)	51.7 (44.1-59.3)	Smartphone add-on
Bogoch et al. 2014	Tanzania	182	STHs					
			A. lumbricoides	99.2	96.4	98.4	98.2	Newton NM1
			T. trichura	93.8	81	97.4	63.0	Newton NM1
Bogoch et al. 2014	Côte d'Ivoire	180	Schistosomiasis, trichuriasis					
			S. mansoni	68.2 (60.1-75.5)	64.3 (35.1-87.2)	95.4 (89.5-98.5)	15.8 (7.5-27.9)	Smartphone add-on

STH = Soil-transmitted helminth
NR = Not reported

Study, author	Study location	Number of samples, *n*	Disease	Sensitivity, % (95%CI)	Specificity, % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)	Technology and/or device used
			T. trichura	30.8 (19.9-43.4)	71 (61.1-79.6)	40.8 (27-55.8)	61.2 (51.7-70.1)	Smartphone add-on
Stothard et al. 2014	Uganda	50	Malaria					
			Plasmodium spp.	93.5 (78.6-99.2)	100 (82.4-100)	100 (88.1-100)	90.5 (69.6-98.8)	Newton NM1
Ephraim et al. 2015	Ghana	49	Schistosomiasis					
			S. haematobium	67.6 (49.4-82)	100 (74.7-100)	NR	NR	Cellscope
			S. haematobium	55.9 (38.1-72.4)	93 (66-99.7)	NR	NR	Foldscope
D'Ambrosia et al. 2015	Cameroon	33	Loiasis (*Loa loa* filariasis)					
			Loa loa	100.0	94.0	NR	NR	Cellscope Loa
Birhanie et al., 2015	Ethiopia	180	Malaria					
			Plasmodium spp (Adults and children)	93.8 (87.1-100)	87.9 (79.7-96.1)	86.4 (77.2-95.5)	94.6 (88.7-100)	Cyscope
Bogoch et al. 2016	Laos	104	Opisthorchiasis					
			Opisthorchis viverrini	70.6 (59.6-79.7)	89.5 (65.5-98.2)	96.8 (87.8-99.4)	40.5 (26-56.7)	Newton NM1
Coulibaly et al., 2016	Côte d'Ivoire	226	Schistosomiasis, intestinal parasites					Newton NM1
			S. mansoni	50 (25.4-74.6)	99.5 (97-100)	85.7 (42-99.2)	97.3 (93.9-98.9)	Reversed-lens CellScope
			S. haematobium	35.6 (25.9-46.4)	100 (96.6-100)	100 (86.7-100)	70.1 (63.1-76.3)	Reversed-lens CellScope
			G. intestinalis	84 (63.1-94.7)	100 (95.2-100)	100 (80.8-100)	96 (89.5-98.7)	Newton NM1
			E. histolytica/dispar	83.3 (36.5-99.1)	100 (96-100)	100 (46.3-100)	99.1 (94.6-100)	Newton NM1
Coulibaly et al., 2016	Côte d'Ivoire	223	Malaria					
			P. falciparum	80.2 (73.1-85.9)	100 (92.6-100)	100 (96.4-100)	65.6 (54.9-74.9)	Newton NM1
Bogoch et al. 2017	Ghana	60	Schistosomiasis					
			S. haematobium	100 (59.8-100)	NR	NR	NR	Novel smartphone-based device
Kamgno et al., 2017	Cameroon	16 259	Loiasis (*Loa loa* filariasis)					
			Loa loa	92.8	99.7 (99.6-99.8)	92.8	99.7 (99.6-99.8)	LoaScope

2.2.3 Principal challenges with current point-of-care digital microscopy devices

A number of different approaches and devices show potential as platforms suitable for digital POC medical imaging, but certain issues remain to be addressed to allow wider-scale clinical implementation. Firstly, the FOV currently achievable with most devices is limited and many systems support only imaging of a single microscopic FOV at a time, thus reducing sensitivity as often significantly larger areas (measuring multiple FOVs) need to be analysed to provide reliable results (Rajchgot et al., 2017). Secondly, practical issues need to be considered to allow usability in field settings. These include solving how the sample is navigated and oriented, hygiene issues (cleaning and exposure to contaminations) and requirement in terms of power and storage of images. Thirdly, even though certain devices can be manufactured extremely cost-efficiently (e.g. the Foldscope for less than one EUR), the devices which seem to be more usable tend to be more expensive (e.g. Newton NM1; costing approximately 650 EUR). Lens-free microscopy is a promising technology, as the construction does not require any lenses, while supporting relatively large FOVs, but challenges still limiting this technology include limitations in spatial resolution, difficulties with imaging of thick specimens (samples need to be placed in close proximity to the sensor) and requirements in terms of illumination and computational resources for the reconstruction of images (Greenbaum et al., 2012). One challenge for multiple systems is the scattering of objects of interest at different focal levels in samples, meaning that depth-level focus adjustment is preferred in samples, and practically all solutions at the moment support imaging in single planes only with few exceptions (Sowerby et al., 2016). Moreover, although the utilization of built-in camera systems in smartphones has many advantages, e.g. by allowing immediate processing of images on the internal memory and utilization of the wireless connectivity of the device, it is limited by the lack of universal standards in hardware and software. The wide range of different built-in image processing algorithms in commercially available camera phones result in image feature variations, such as artificial digital sharpening, halos around high-contrast structures and other image post-processing modifications, calling for strategies to increase uniformity (Skandarajah et al., 2014).

In conclusion, during the last decades there has been a gradual shift from purely optical to digital systems in microscopy diagnostics, but the adaptation has been slow and mainly limited to larger laboratories. A number of solutions that enable digitization of microscopy samples with small-sized, inexpensive portable digital microscopy devices have been described during recent years. Smartphone camera optics have been used in many devices either directly or in combination with other optoelectronic components to enable the imaging of samples. To date, the majority of these devices have been studied for field diagnostics of infectious diseases; mainly NTDs, such as STHs and schistosomiasis, malaria and tuberculosis, by visual identification of pathogens in the digitized samples. Although the spatial resolution of these devices in general is lower than what is achievable with high-end systems, multiple studies show that the imaging performance is sufficient for diagnostics of a range of diseases, thus suggesting potential as platforms for POC digital microscopy especially in low-resource settings.

2.3 Computer-aided microscopy diagnostics

The potential to facilitate and automatize image-based medical diagnostics, such as the analysis of microscopy samples, with digital methods has been studied for decades (Al-Kofahi et al., 2010, Voulodimos et al., 2018). As a general term, *computer vision* (CV) is often used to refer to the field of computational methods used to process and analyse digital images and extract useful information from them. Digital image analysis is one type of computer-aided diagnostics (CAD), which has recently received an increased interest for medical applications. When applying digital image analysis methods for microscopy diagnostics, the aim is usually to mimic the tasks of the visual sample assessment, performed by a human observer. Historically, considerable efforts have been made to automatize the analysis of microscopy samples and extract useful data from digitized samples. Much work has been focused on analysing histological samples, scanned with laboratory-grade slide scanners, for assessment of e.g. overall tissue morphology (Al-Kofahi et al., 2010), quantification of histological stainings (Tuominen et al., 2010) and features of prognostic value (Veta et al., 2015, Xu et al., 2016). Digital image analysis methods have also been applied to facilitate diagnostics of infectious diseases by e.g. automatizing detection of malaria parasites (Linder, N. et al., 2014, Poostchi et al., 2018), also with high-end equipment, but not in the same magnitude as analysis of histological samples.

2.3.1 Machine learning and artificial intelligence for image analysis applications

During the last decade, the field of digital image analysis has significantly evolved with the rise of machine learning (ML) and so-called artificial intelligence (AI) based on deep neural networks (Lee, L. I. T., Kanthasamy, Ayyalaraju & Ganatra, 2019). ML refers to the process where computer algorithms learn to perform specific tasks without explicit instructions, by learning from patterns in training data. ML algorithms have been widely implemented for numerous applications, ranging from e-mail spam filters and speech recognition software to object detection and image classification. AI in general refers to the field of computer science where a machine attempts to emulate the decisions of an intelligent human, and ML represent one approach to developing AI systems, where the system 'learns' to make decisions based on patterns in training data. Numerous methods exist to developing AI algorithms for image analysis applications, with deep learning currently representing one widely used method for image-based medical diagnostics (Figure 3). Thus, in scientific literature, AI is a loosely defined term to describe automated systems that can perform tasks considered to require "intelligence", and deep learning a more precise term for ML techniques that utilize multi-layered arithmetic operations (Liu et al., 2019). Currently, AI in medical image analysis is used to refer to the automatized analysis of medical images with various algorithms based mainly on artificial neural networks.

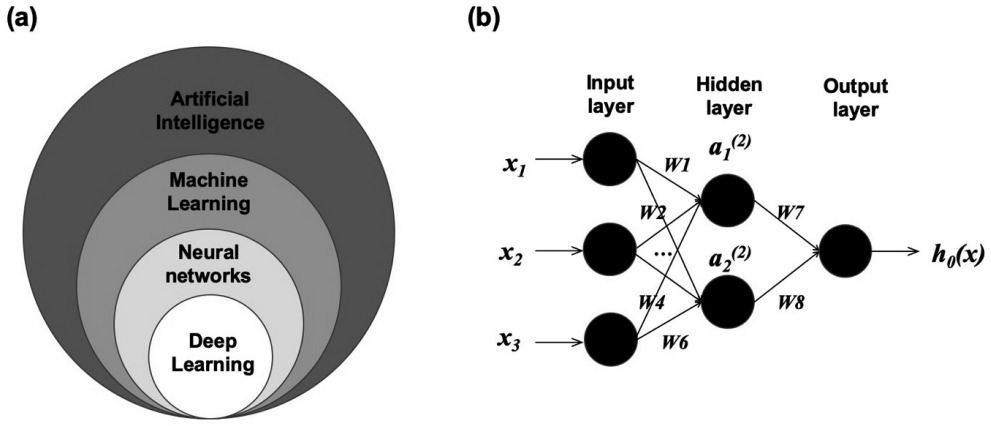


Figure 3. Relationships between artificial intelligence (AI) and machine learning (ML) with deep neural networks. Schematic image showing **(a)** context of AI, ML and deep learning with neural networks, and **(b)** overview of the structure of a simple artificial neural network. In **(b),** the synapse signal x (input) represents a number, and the output of each individual neuron is calculated by a non-linear function based on the sum of inputs, using weights (W) to increase or decrease the forwarded signal strength. The information is processed through layers, typically including multiple hidden layers, to the output layer which outputs the value of the network.

During the last decade, the interest in deep-learning based AI for digital pathology has increased significantly, following research breakthroughs in the field and the increased computational power (particularly more powerful GPUs) of modern computers (Krizhevsky, Sutskever & Hinton, 2012, Bera et al., 2019). Deep-learning algorithms have been recognized as an efficient approach to extracting complex data from medical images, with the performance of modern algorithms even superseding the levels of human experts for certain applications (Cireşan, Meier & Schmidhuber, 2012, Gulshan et al., 2016, Esteva et al., 2017).

2.3.2 Architecture and principles of deep neural networks for image processing applications

Deep-learning algorithms are biologically inspired, multi-layered arithmetic computational systems, which consist of so-called artificial neural networks (ANNs; named due to resembling the structure of neurons in the visual cortex). The interconnected nodes in ANNs process and modify input data and forward the modified information to higher layers to eventually yield an output value, based on the analysed data (Figure 3b). Simple ANNs have been studied since the 1950s, and certain fundamental principles of modern algorithms (such as backpropagation) were developed already during the 1980s (Goodfellow, Bengio & Courville, 2016). Compared to how information is processed in "traditional" computers, neural networks have certain advantages, such as being able to process significant amounts of information simultaneously (due to the large numbers of individual neurons), and being able to both store and process information without the need for external memory. Due to this, to achieve the maximum capacity of complex algorithms, hardware that supports efficient parallel processing (i.e. GPUs that can process multiple tasks simultaneously) is required (Lundervold, Lundervold, 2019). A basic (feedforward) neural network consists of layers of interconnected nodes with adaptive parameters, called weights (Figure 3b), which are summed up in the so-called linear combination of inputs to the neurons. The result of this calculation is consequently passed through an activation function, which determines the output of the neuron, which again is forwarded to the neuron in the next layer. The activation function is usually a non-linear function, such as the sigmoid function which outputs a range of values between 0 and 1, which are then forwarded to the following layer which further processes the information. This multi-layered structure is the basic for deep learning, where the output of certain layers is forwarded as the input for the following to allow complex processing of information in large networks with (usually) large numbers of hidden layers. Convolutional neural networks (CNNs) are a sub-category of deep-learning networks that have proven to be especially suitable for image processing (Anwar et al., 2018). CNNs utilize so-called convolutional layers that detect certain features in the input image (such as edges, patterns or colour intensities), regardless of locations in the image (using so-called feature maps with shared weight matrices in the units of the layer). This is a fundamental principle that allow the

detection of higher-level image features which would otherwise require vast amounts of layers and combinations of weights to detect all possible orientations and locations of objects in the input data. Convolutional layers are typically placed in the bottom layers of the network to process raw input data (such as pixels) and can often be trained with unsupervised learning (i.e. without a pre-decided prediction task) to detect generic features that are applicable for multiple tasks ('pre-trained' networks and 'transferred learning'). Another essential feature of CNNs is pooling, which is a type of non-linear down-sampling to partition the input data (image) into smaller regions and reduce the number of parameters to decrease the required computation (and risk for overfitting). Other commonly utilized CNN features include rectified linear units (ReLUs), which are activation functions that increase the nonlinear properties the network, and 'fully connected layers' to perform higher-level classification or reasoning in the network (Goodfellow, Bengio & Courville, 2016) (Figure 4). After processing of the information through the layers of the network, the final output is obtained from a subset of neurons in the so-called output layer.

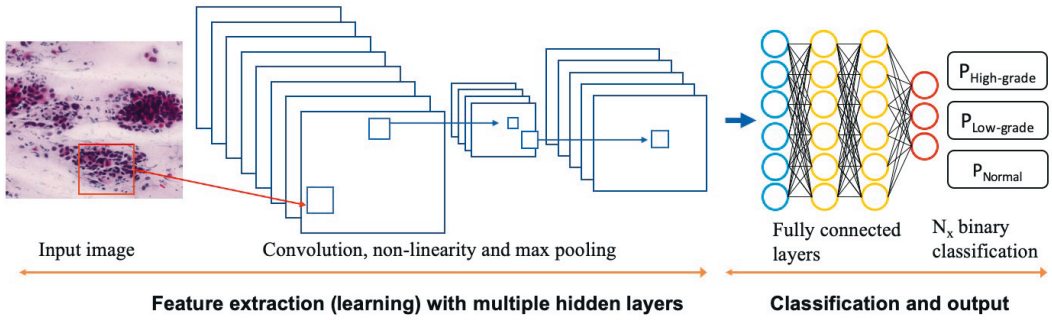


Figure 4. Schematic view of image classification task with a convolutional deep neural network. Images illustrating the input image being fed into the network, where the information is processed in the different layers before the output layer returns the class which mostly resembles the "learned" features on the network.

The "learning" and "training" of the networks occur when the individual weights are adjusted with an optimization algorithm (the gradient descent) based on a function measuring the correctness of the output (i.e. minimizing the 'loss' by adjusting the weights in the network, based on the difference between the target value and predicted value). As the number of individual weights can be significant (even billions in complex networks), this task often requires significant computational power. In this way, deep learning

algorithms can be trained and applied for highly-efficient patter-recognition tasks in complex images, such as for image-based medical diagnostics (Lundervold, Lundervold, 2019). These principles also explain why methods utilizing deep learning models are more suitable for certain types of tasks, such as analysing of large volumes of data and detection of patterns, and also associated with certain limitations, which is discussed in more detail in the separate section on this topic (2.3.5).

2.3.3 Digital microscopy diagnostics with deep-learning based artificial intelligence

The main goal with the current generation of automatized deep neural network algorithms for medical diagnostics, or commonly referred to as 'medical AI' algorithms, is to create clinically useful pattern recognition tools. To date, this type of algorithms (or 'models') has been applied and studies especially in image-based fields of medicine, such as pathology (Bera et al., 2019), dermatology (Esteva et al., 2017), ophthalmology (Ting et al., 2017), radiology (Lee et al., 2017) and cardiology (Zhang et al., 2018). In pathology, the technology has been studied extensively for the analysis of histological samples, such as the detection of breast cancer lymph node metastases (Ehteshami et al., 2017), tumor grading (Ertosun, Rubin, 2015) and classification of tissue types (Chen et al., 2017). Furthermore, the technology has also been applied for the detection of various infectious pathogens, e.g. malaria parasites in digitized thin blood smears in laboratory settings (Poostchi et al., 2018). Compared to visual microscopy which is prone to subjectivity (Stoler et al., 2001, Bigras et al., 2013), digital methods can provide more objective and reproducible results. Ideally, AI models yield completely reproducible results, and could therefore be usable to reduce subjectivity and intra-observer variability (Bera et al., 2019). For certain applications, studies have described how state-of-the-art deep learning algorithms can reach even superhuman levels of performance, e.g. in the detection of diabetic eye disease (Gulshan et al., 2016) and classification of skin lesions (Esteva et al., 2017). Furthermore, deep learning-based analysis of samples seem to allow extraction of information from digital samples not previously accessible to human experts (as in the field of Computational Pathology), to e.g. predict survival for patients based on tissue morphology (Bychkov et al., 2018, Wang et al., 2017, Fu et al., 2019) and extract data not previously considered to be quantifiable from

image-based data - such as age or blood pressure from retinal images (Poplin et al., 2018). From a clinical perspective, studies from recent years highlight how this type of medical deep-learning AI's can augment the human expert to improve performance when used together with conventional protocols, thus suggesting usability as diagnostic support-tools for clinicians (Steiner et al., 2018). Notably, in 2018, the first 'medical AI' received approval for clinical use by the FDA for the detection of diabetic retinopathy (FDA, 2018).

2.3.4 Digital image analysis applied to point-of-care microscopy diagnostics

Although digital image analysis with conventional computer vision- and deep learning-based methods have been applied for the analysis of a wide variety of samples in laboratory conditions, less work has been conducted on the analysis of samples digitized at the POC in more rural settings. To date, the majority of work on automatized POC digital microscopy has focused on facilitating the diagnostics of infectious diseases, such as neglected tropical diseases and malaria (Saeed, Jabbar, 2017; Vasiman, A., Stothard, J.R. & Bogoch, I.I. 2019). In an early stage, Breslauer et al. described as a proof-of-concept how tuberculosis bacilli could be visualized in digital images captured with a smartphone connected to a microscope and identified using a pattern recognition algorithm (Breslauer et al., 2009). Using a similar software solution, Linder et al. applied pattern-recognition algorithms for the detection of *S. haematobium* ova in digital images captured with the camera module from a web-camera, and achieved overall a high specificity and moderate sensitivity (Linder et al., 2013). Efforts have been made to facilitate malaria diagnostics by automatized detection parasites, such as in the work by Rosado et al. where the detection and classification of *Plasmodium* parasites in Giemsa-stained blood smears with a pattern-recognition algorithm, running on a smartphone was described with promising result (Rosado et al., 2017). Following this, newer generations of algorithms based on deep neural networks have been applied to detect malaria parasites in digital samples, such as in a smartphone-based system, where a reasonably high diagnostic accuracy of 91% was achieved (Oliveira et al., 2017). Notably, in 2018 an "AI-laboratory" was announced at Makerere University in Uganda for research around digital smartphone-based diagnostics of malaria (Lewton T., McCool A, 2018); but apart from smaller-scale academic studies, the clinical adaptation of these techniques has been relatively slow.

2.3.5 Principal challenges and limitations with clinical adaptation of the current artificial intelligence algorithms

Although modern deep-learning algorithms can be highly efficient for applications in image-based medical diagnostic, they are associated with certain challenges that need to be acknowledged before wider-scale clinical implementation (Kelly et al., 2019). To develop reliable algorithms, substantial amounts of high-quality and manually-labelled training data is required to accurately train the networks (Tizhoosh, Pantanowitz, 2018). The annotation of training data is a crucial step, and is therefore ideally performed by experts, but is often both labour-intense and time-consuming as the required number of samples is typically substantial. As the algorithms "learn" entirely based on training data, the performance of the system highly mimics that of the experts providing the training data; thus, potentially including pre-existing bias and noisy data (Gianfrancesco et al., 2018). So-called unsupervised machine learning can partly circumvent this by classifying information and patterns in data without the need for human labelling, but on the other hand requires higher computational resources and generate results which are more difficult to interpret (as the detected patterns are not pre-defined) (Arevalo et al., 2015). Another problem in the development of algorithms is the limited availability of public data sets and standardized benchmarking procedures, which make external validation of algorithm performance challenging. This is important as overfitting (i.e. meaning that the model is trained to predict the training data 'too' accurately and does not generalize to new data) is a common problem with deep learning models. Notably, efforts have recently been made to create international evaluation procedures for medical AI's (Wiegand et al., 2019). From a technical point of view, the analysis of medical image data, such as high-detailed WSIs, typically require processing of image files with extremely large dimensions (gigapixel images), meaning that storage and processing quickly become demanding in terms of computational resources (Campos et al., 2017). Lastly, even though encouraging results have been achieved with AI algorithms for medical applications, the clinical adaptation has been slow. Potential reasons for this include that automatized digital algorithms typically classify data in a simplified, categorical way, compared to manual analyses which often is more analytic and probabilistic (Pena, Andrade-Filho Jde, 2009; Harrel, 2018). As the

algorithms also generate results via relatively unobservable methods, this perceived "black box" nature may also impede the clinical adaptation (Maddox, Rumsfeld & Payne, 2019).

In conclusion, digital methods have for decades been studied as potential tools to facilitate traditional microscopy diagnostics by e.g. detecting, classifying and measuring objects of interest, to reduce the workload for clinicians and improve reproducibility. Currently, 'AI' methods, based on deep learning with artificial neural networks, show potential as efficient tools for complex medical image analysis tasks, such as the analysis of microscopy samples, and are likely to highly impact fields of image-based medical diagnostics. Digital image analysis algorithms have been studied extensively for e.g. analysis of histological samples in laboratory settings, but have not yet been widely adopted in clinical practice, nor studied extensively for POC diagnostics in rural settings.

2.3 Breast cancer

Breast cancer is the most common form of cancer in women and the second leading cause of cancer-related death among women globally (Siegel et al., 2019). The incidence of breast cancer has been rising for the last decades in most countries, and is estimated to increase especially in Africa, Asia and South America due to an increased proportion of the elderly population (Bray, F., Moller, 2006). When diagnosed at an early stage, breast cancer prognosis is significantly more favourable than that of more advanced cancers (Boyle, 2003), with the five-year survival for patients with localized breast cancer being 97%, compared to 27% for advanced diseases (Siegel et al., 2019). In low-resource areas where the access to healthcare services is limited, initial diagnosis is often delayed, resulting in many cases still being discovered at late stages (Ngowa et al., 2015). The recommended treatment for most breast cancer patients involves surgery, with the type of surgery (e.g. breast-conserving or mastectomy) determined individually by the extent of the disease while considering patient-specific factors (preference, cosmetic factors). The need for adjuvant radiation therapy, hormonal therapy and chemotherapy is decided based on tumor characteristics (histological features) and patient epidemiology (e.g. age and menopausal status). Although the incidence of breast cancer is increasing, overall mortality has decreased, likely due to more efficient treatment methods with the possible contribution from increased mammography screening being a subject of debate (Cronin et al., 2006, Jørgensen et al., 2017). Anatomically, the spread of breast cancer, and other cancers, is most commonly classified according to the international TNM classification system (currently the 8th edition from 2016), originally developed by the Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC). The TNM classification system includes three main parameters; T for size and extension of primary tumor, N for degree of spread to regional lymph nodes and M for presence of distant metastases. In addition to this, other parameters can also be included, e.g. grade (G), elevation of serum markers (S), invasion into lymphatic vessels (L), and prefix modifiers to indicate based on what the classification is made (e.g. ‘c’ for staging determined prior to treatment, or ‘p’ for staging by pathological examination of surgical specimens). Based on the TNM system, the AJCC staging system is widely used clinically to classify cancers, and incorporates further information also about additional tumor features, such as histological grade and expression of hormone receptors (ER and PR) and oncogenes (HER2) (Amin et al., 2017).

2.3.1 Breast cancer histopathology

Histological classification of breast cancers is usually performed according to the World Health Organization Classification criteria (Elston, Ellis, 2002), using the entities *in situ* carcinoma, invasive ductal carcinoma (the most common invasive form), invasive lobular and breast carcinoma of special types. Tumor differentiation is generally performed using the Bloom - Richardson grading method based on factors such as mitotic count, nuclear pleomorphism and tubule formation (Bloom, Richardson, 1957). To determine tumor characteristics, the expression of certain molecular biomarkers such as estrogen receptor (ER), progesterone receptor (PR), cell proliferation (Ki-67) and human epidermal growth factor receptor 2 (HER2) are routinely assessed in tumor samples. This type of molecular typing is essential in decision-making on targeted therapies, where for example tumors expressing hormone receptors ER and PR indicate that the patient may benefit from hormonal therapies (e.g. tamoxifen and aromatase inhibitors) (Harvey et al., 1999), and expression of the HER2 protein supports the usage of HER2-targeted therapies (antibodies). Most breast cancers are positive for both hormonal receptors (ER and PR), and a minority (20%) are negative for both (Bauer, Parise & Caggiano, 2010). The expression of biomarkers, such as ER, are typically assessed by microscopy analysis (scoring) of immunohistochemically (IHC) stained tumor sections (Hammond et al., 2010). This method is prone to subjectivity with significant degrees of intra- and interobserver variability being reported, especially in the analysis of low-grade positive samples (Mann et al., 2005; Hede, 2008). As the ER-scoring of a significant amount of samples (up to 20%) has been estimated to be possibly inaccurate, the threshold for classification of a sample as ER-positive was lowered from >10% to >1% (cells expressing ER) in guidelines from 2010 to more reliably identify patients likely to benefit from endocrine therapy (Hammond et al., 2010).

2.3.2 Intraoperative breast cancer histopathology

The assessment of histological samples during the surgery procedure plays an important role in a number of surgical procedures. One example is the detection of breast cancer axillary metastases during the surgery; a process which is essential in disease staging and

affects both treatment and prognosis (Fisher et al., 1983). Axillary nodal status correlates with tumor size and is considered an important prognostic factor of recurrence and survival. Presence of axillary metastases often indicates a need for more extensive surgery, traditionally in the form of axillary lymph node dissections (ALND) or more recently, lesser axillary surgery, such as targeted axillary sampling (i.e. limited or partial ALND) (Ditsch et al., 2019) and regional radiotherapy (Cardoso et al., 2019). The assessment of axillary lymph node status is therefore important both for disease staging and to avoid unnecessarily extensive treatment interventions with the associated morbidity and complication risks for node negative patients (Cardoso et al., 2019, Samphao et al., 2008). Microscopy evaluation of sentinel lymph nodes using the frozen section (FS) procedure is a common method to determine axillary lymph node status. The FS technique involves rapidly freezing the tissue samples during the operation, cutting the specimens into thin sections with a cryostat and staining the samples for microscopy analysis. The staining is usually performed with methods such as rapid haematoxylin and eosin (H&E) or toluidine blue for overall morphology, and rapid IHC-staining for cytokeratins. Toluidine blue is a metachromatic dye which is widely available and relatively inexpensive, and due to its acidophilic properties binds to proteins and areas rich in DNA and RNA, such as dividing cells (Sridharan et al., 2012). Cytokeratins (CK) are the dominant intermediate filaments in epithelial cells, and IHC-staining for CKs is typically performed to visualize cells of epithelial cell lineage, such as carcinomas, by binding to multiple common CKs ("pancytokeratin" staining) (Painter et al., 2010). Although the tissue quality achievable with FS is inferior to traditional histological methods, samples can be prepared and analysed significantly faster than with conventional methods - typically in less than 20 minutes (Novis, Zarbo, 1997). The FS procedure is considered accurate for the detection of larger metastases (macrometastases over 2mm in size), but not as sensitive for smaller lesions (micrometastases and isolated tumor cells), and is also prone to a certain degree of subjectivity (Yeh et al., 2015). Notably however, the significance of occult isolated tumor cells and micrometastases in low axillary disease burden (in terms of surgical management and patient outcome) appears to be negligible (Cardoso et al., 2019). Furthermore, FS assessment requires the presence of a pathologist on-site (or close to the site) to analyse the samples. To solve this in areas without access to on-site pathologist services, virtual microscopy with remote assessment of samples by a pathologist has been tested and already clinically implemented at certain locations (Thorstenson, Molin & Lundstrom, 2014). Studies suggest the interpretation of FS

WSIs is comparable to conventional microscopy (Gifford et al., 2012), but currently requires access to high-end equipment for the digitization process. Of all methods for intraoperative assessment of tissue samples to prevent reoperation (e.g. touch preparation cytology, intraoperative ultrasonography and micro-computed tomography), FS is the most reliable (Ko et al., 2017). Thus, in locations where access to pathology services on-site is limited, post-surgery findings can warrant repeated surgical interventions, which could otherwise have been prevented (Aziz et al., 2006). Due to the high reoperation rate (38%) after breast conserving surgery, digital microscopy at the POC for cancer surgeries has been proposed as one potential solution to this (Morrow et al., 2009, Evans et al., 2009).

2.4 Cervical cancer

Cancer in the cervix is globally a common cancer in women. In 2018, it was the most commonly diagnosed cancer in women in 28 countries, and the deadliest in 42 (Bray, Freddie et al., 2018). The vast majority of cases of cervical cancer occur in low- and middle-income countries (Parkin et al., 2008). According to current understanding of the aetiology, the disease is almost exclusively caused by persistent infections of high-risk human papillomavirus (HPV) strains which causes progressive dysplasia in the cervical epithelium (Bosch et al., 2002). In total, over 100 types of HPV are known, of which at least 14 are oncogenic, with HPV type 16 and 18 causing the majority (>60%) of cancers. HPV is the most common sexually transmitted disease and affects approximately 80% of the global population at some point in their life, with the initial infection usually occurring during adolescence or early adulthood. The majority of HPV infections regress spontaneously within 18-24 months but in a minority (3 - 5%) the infection persists, and causes cellular changes with the risk of progressing into invasive cancer. Cervical cancer is a highly preventable disease (World Health Organisation, 2014). The current standard screening method for cervical dysplasia is the visual microscopy examination of cervical cytology samples, stained using the Papanicolaou staining method (Pap smear analysis). Pap smear screening has drastically reduced the cervical cancer incidence and mortality in areas where routine screening is implemented (Saslow et al., 2002). This screening method involves visually examining the morphology of cervical cells in samples prepared either using the conventional Pap smear technique (cervical smears) or using so called liquid-based cytology (LBC). For LBC, the sample preparation is performed with automatized sample processors (e.g. ThinPrep processors), which disperse the cells into even monolayers on the microscope glass and remove excess debris in the process, whereas conventional Pap smears can be prepared by applying the unprocessed cervical sample directly on the glass. Regardless of method, the final analysis of sample is performed by a pathologist who evaluates the cellular morphology and report detected atypia. Findings are typically reported using the standardized Bethesda classification system (Solomon et al., 2002), where findings are graded from low (e.g. low grade squamous intraepithelial lesion; LSIL) to high grade (e.g. high grade squamous intraepithelial lesion; HSIL), depending on degree of dysplasia (Figure 5) and cellular lineage (e.g. squamous or glandular). Lower-grade lesions have a high chance

of spontaneous regression, and can usually be controlled in 6-12 months, whereas higher grade changes indicate a need for more rapid intervention, initially often by referral to colposcopy (Wright et al., 2002). Pap smear screening is recommended at 3-5 years intervals, depending on individual risk factors for the patient (e.g. co-infection with HIV), and when adequately performed, reduces the risk for cervical cancer by up to 80% (Koliopoulos et al., 2017). Recently, HPV-DNA testing has been established as an alternative screening method with high sensitivity (Ogilvie et al., 2018) but lower specificity, compared to repeat cytology (Koliopoulos et al., 2017). Moreover, effective vaccines against HPV are now commercially available, but despite increased vaccination coverage, screening methods will remain necessary for the foreseeable future as vaccines are not effective against pre-existing HPV infections. Thus, even though vaccinations have the potential to virtually eradicate the disease in the future, the benefits of even the most efficient vaccination programs will not be realized for decades (Randall, Ghebre, 2016). Due to the low specificity of the HPV DNA test (most infections do not progress to cancer), there is also a need for a triage test for positive women (Bray et al., 2018). During the next decade, the cervical cancer incidence is predicted to rise and the amount of yearly deaths expected to double, with the largest burden of disease in sub-Saharan Africa (Mboumba et al., 2017). As screening coverage here is severely lacking in many areas, cervical cancer remains the leading cause of cancer-related death among women (Bray, Freddie et al., 2018). A major challenge here is the limited access to laboratory infrastructure and pathologists, with often less than one practising pathologist per one million inhabitants (Nelson et al., 2016). Notably, considerable efforts have been made during the last decades to automatize the screening process of cervical smears, but the development of reliable, cost-efficient systems capable of fully-automatized analysis of whole slides (i.e. not individually segmented cells) has been challenging (William et al., 2018, Z. Lu et al., 2017), likely due in part to the complexity of this type of samples (Bengtsson, Malm, 2014).

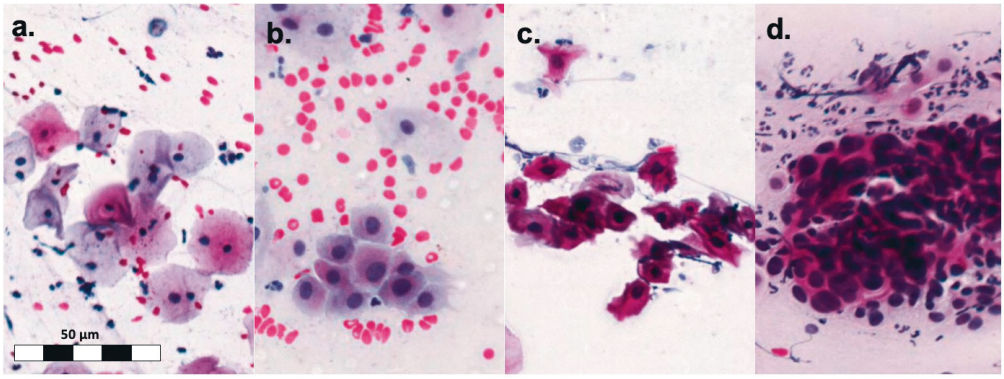


Figure 5. Squamous cervical-cell atypia of various degrees. Image showing squamous-cell atypia, classified according to the Bethesda system; with normal epithelium **(a)**, atypia of undetermined significance (ASC-US) **(b)**, low-grade (LSIL) **(c)** and high-grade atypia (HSIL) **(d)**.

2.5 Neglected tropical diseases

The neglected tropical diseases (NTDs) is a diverse group of major, disabling chronic infections that are especially common in tropical and subtropical low-resource areas with poor sanitation (Hotez et al., 2007). Compared to diseases such as HIV/AIDS, malaria and tuberculosis, significantly less resources are dedicated towards fighting these diseases (Payne, Fitchett, 2010). In 2017, the WHO recognised 17 diseases as NTDs, although there is some debate about specifically which diseases belong to the group (Payne, Fitchett, 2010). On a global scale, the NTDs affect over one billion people and represent significant socioeconomic burdens for developing economies (Molyneux, Hotez & Fenwick, 2005); https://www.who.int/neglected_diseases/diseases/en/). The most prevalent NTDs are caused by parasitic worms, of which the most common are the soil-transmitted helminths (STM) *Ascaris lumbricoides, Trichuris trichiura* and hookworms, and Schistosomes (*Schistosoma mansoni* and *Schistosoma haematobium)*. These poverty-related diseases are more common in children, where they result in impaired development and growth, neurocognitive problems and diminished physical fitness (Crompton, Nesheim, 2002) and represent significant causes of maternal morbidity and pregnancy complications (Christian, Khatry & West, 2004, Brooker, Hotez & Bundy, 2008). Globally, more than 415,000 annual deaths are caused by NTDs (Hotez et al., 2006). An important method of disease control for a number

of NTDs has been the coordinated administration of preventive chemotherapy in mass-drug administration programs (MDAs). MDAs refer to the mass-treatment of individuals in communities at risk to reduce the pool of infection and proliferation of parasites. The implementation of MDAs has been boosted through WHO by partners in the pharmaceutical industry since 2012 (Uniting to Combat NTDs, 2012), and in 2015 over one billion people received preventive chemotherapy for NTDs with drugs such as praziquantel, albendazole and mebendazole, representing significant progress towards achieving the WHO Roadmap goals to overcoming the global impact of NTDs (World Health Organization, 2012). Apart from improving levels of hygiene and sanitation and organisation of MDAs, improved access to early diagnosis with better POC diagnostic solutions are vital component in disease control and monitoring of NTDs (World Health Organization, 2017). A requisition for programs addressing NTDs is cost-efficient, easy-to-use and robust diagnostic tests which are deployable in low-resource settings (PATH, 2015). When infection levels are reduced through successful disease-control programs, the importance of these tests is especially pronounced to enable fast and accurate diagnosis and monitor disease prevalence. While significant progress has been made in the availability of NTD treatments during recent years, little progress has been achieved in the development of improved diagnostic techniques; likely due to the perceived lack of viable commercial markets (Peeling, Rosanna W., Boeras & Nkengasong, 2017). The current golden standard for diagnostics of STH and schistosomiasis rely on light microscopy examination of microscopy samples to detect and count the number of parasite eggs that are excreted in stool or urine. For STH, samples are typically prepared using the Kato-Katz method to visualize parasite ova (Knopp et al., 2008). Strengths with the Kato-Katz methods include simplicity of sample preparation, low per-sample preparation costs and few false positives. The tests can furthermore be used to detect multiple different parasite species at the same time (Lamberton, Jourdan, 2015). Conventional diagnosis of *Schistosoma haematobium* relies on microscopy examination of sedimented or filtrated urine samples to detect parasite eggs (Knopp et al., 2013). These techniques have been extensively used and validated worldwide and are generally regarded as having a high specificity, but limitation of the techniques include the need for trained microscopists on-site and reduced sensitivity especially in low-burden infections (Nikolay, Brooker & Pullan, 2014). Visual microscopy sample analysis to detect and quantify parasites is furthermore both a time-consuming and laborious procedure; requiring up to 10 minutes for a skilled microscopist to reliably analyse a single glass slide (Speich et al., 2010). As by

far the largest burden of disease of NTDs is in low-resource areas with limited access to laboratory services, these diseases are still severely underdiagnosed due to lack of resources for diagnostics (Bethony et al., 2006).

3 AIMS OF THE RESEARCH

The aims of this doctoral thesis were to study areas of applications for point-of-care digital microscopy diagnostics, supported by computer-aided diagnostics and deep-learning based artificial intelligence, with emphasis on potential applications in low-resource environments.

Specifically, the aims were to:

1. Study the digital assessment of hormone receptor (ER) expression in breast cancer samples, digitized with a low-cost digital microscope, and compare the results to visual assessment and analysis of samples from a high-end slide scanner.
2. Evaluate if low-cost, point-of-care digital microscopy can be used to digitize sentinel lymph node frozen sections for remote detection of breast cancer metastases, compared to digitization with a traditional high-end system.
3. Develop, implement and evaluate a digital diagnostics system for cytological screening for cervical cancer, where slides are scanned at the point-of-care and analysed with a deep-learning system to detect squamous-cell atypia.
4. Develop and evaluate a deep-learning based, point-of-care digital microscopy system for diagnosis of the most common neglected tropical diseases.

4 MATERIALS AND METHODS

4.1 Study specimens

4.1.1 Breast cancer tissue microarrays (I)

For Study I, we acquired a total of 193 archived human breast cancer tissue samples. The samples were arranged into tissue microarray slides (TMAs), and individual samples were verified as representative breast cancer tissue by a certified pathologist by light microscopy examination. Of these samples, 135 were selected from the FinProg Breast Cancer Database. The Finprog nationwide patient cohort includes tissue samples and follow-up information on women diagnosed with breast cancer in 1991-1992 in Finland, and is publicly accessible online (www.finprog.org). The remaining 58 samples originated from the Predect series of breast cancer samples (www.predect.eu; predect.webmicroscope.net). Predect is an Innovative Medicine Initiative (IMI) collaboration of the European Union which includes academic and biotech laboratories and partners in the pharmaceutical industry. The tissue samples analysed were prepared from formalin-fixed paraffin embedded (FFPE) samples, which were acquired from the archives of the diagnostic pathology laboratory. The FFPE samples were punched with a 0.6mm needle and transferred into TMA-blocks which were cut into 5µm sections and IHC-stained for ER using mouse monoclonal anti-ER primary antibodies. Visualization of staining was performed with the 3–3'-diaminobenzidine (DAB) chromogen and tissue counterstaining performed using haematoxylin.

Ethical approval for this study was issued by the Central Laboratory for the Hospital District of Helsinki and Uusimaa, HUSLAB, the Ethical Committee of Surgery of the Hospital District of Helsinki and Uusimaa (No. 94/13/03/02/2012) and The Ministry of Social Affairs and Health (No.123/08/97). According to the Ministry of Social Affairs and Health, Finland Act on the Medical Use of Human Organs, Tissues and Cells (Amendments up to 277/2013 included), written informed consent was not required as no clinical records were retrieved and the study contained no personal identifiers.

4.1.2 Breast cancer sentinel lymph node frozen sections (II)

For the samples in Study II, we acquired routinely collected sentinel lymph node frozen sections from breast cancer surgeries at hospitals within the Hospital District of Helsinki and Uusimaa in southern Finland. The samples had been prepared according to local standard operating procedures during the year 2016, and archived in the files of the pathology laboratory of the hospital district (HUSLAB, Helsinki, Finland). Samples were collected during breast cancer surgeries in the hospital region, rapidly frozen and cut into 5 µm thick sections and consequently stained according to routine protocols. Frozen sections were stained with toluidine blue for overall tissue morphology and rapid pancytokeratin IHC-stained. In total we identified and acquired samples from 80 breast cancer patients for this study. Of these, 28 patients were node positive (i.e. histologically verified visible tumor cells in the samples), and correspondingly 52 patients were node negative (i.e. had no detectable tumor cells in the samples). The majority of patients had sections stained using both staining techniques available, but for a minority only one staining was used (i.e. toluidine blue or IHC staining). We included one slide for each staining type available per patient for the analysis, and an area of representative tissue from each slide was selected by conventional light microscopy assessment by an experienced pathologist.

This study used archived, routinely collected tissue samples, obtained during breast cancer surgeries during a period of one year. The study was approved by the scientific and ethical committee of the Helsinki Biobank (HUS/359/2017, September 18, 2017). Written consent was not required as the study material and images contained no personal identifiers, and since the study was retrospective, in accordance with the Ministry of Social Affairs and Health, Finland Act on the Medical Use of Human Organs, Tissues and Cells (Amendments up to 277/2013 included).

4.1.3 Cervical cytology samples (III)

The samples in Study III were routinely collected cytological smears (Pap smears), collected from women attending the HIV control program at a rural clinic in Kenya (Kinondo Kwetu Health Services, Kinondo, Kwale County, Kenya) (Figure 6). The patients were volunteering, non-pregnant women, aged between 18 and 64 (mean age: 42.0) who had previously been confirmed positive for HIV, and provided signed informed consent. In total, 740 samples were collected for this study. Samples were acquired by nurses who had previously received training for Pap smear acquisition. The sample were collected using a conventional cervical broom sampling kit after which fixation and staining was performed at the site according to Papanicolaou-staining protocols (Gill, 2013).

Figure 6. Study site and point-of-care digital laboratory at research site in Kinondo Kwetu Health Services clinic. Images showing nurses with sample acquisition equipment **(a)**, slide staining equipment **(b)** with fume hood at slide staining bench and digitization and uploading of Pap smear with the point-of-care slide scanner **(c)**.

Cytological samples were applied to clean frosted glass slides, which was fixated with the provided fixative solution. Following air drying, the slides were then further fixed in 95% ethanol, and transferred through an alcohol series (70% and 50%) and rinsed with distilled water. Next, staining was performed with Meyer's haematoxylin, after which the slides were washed with distilled water and again submerged in 95% ethanol. Counterstaining was performed with the OG-6 and EA-65 staining solutions and slides were submerged between the steps in 95% ethanol. Dehydrating was carried out with absolute ethanol, and sample clearance using Rectified Xylene, after which slides were covered with

coverslips with the diethyl propene xylene (DPX) mounting medium. Stained slides were places in a level position and allowed to air-dry at room temperature overnight before analysis. Stained samples were stored in slide boxes before digitization, after which they were transported to the pathologist at the pathology laboratory in Mombasa (Coast Provincial General Hospital, Mombasa, Kenya) for visual assessment.

Ethical approval was issued for the study by the Ethical Review Committee at the National Commission for Science, Technology and Innovation (Pwani University, Nacosti, Kenya) (No. ERC/PU-STAFF/005/2018). Research work for the study was also approved by the Helsinki Biobank (No. 359/2017). Eligible patients who fulfilled the inclusion criteria were provided with information both orally and in written form about the study purpose and Pap smear procedure. Information was provided in English and Swahili, and signed consent acquired from participants prior to study participation. Patients were reimbursed for travel expenses by the study organizes, but not offered other monetary compensation for participation in the study. In case of abnormal test results, the patients were referred to a gynaecologist for adequate further medical interventions and all treatment expenses were covered by the study organizers. We used the secured, password-protected web-based data collection platform REDCap (Research Electronic Data Capture, Vanderbilt University, Nashville, TN, USA) to collect and store patient information. The software was running on an encrypted and password-protected local server, stored in a locked room, which was accessible only to study personnel. Paper forms containing patient information were stored in locked cabinets in a locked room, separated from the rest of the clinic for the duration of the study. All samples (digital samples and glass slides) were pseudonymized using study numbers and no personal identifiers or patient information were uploaded to the cloud-server.

4.1.4 Parasitological samples (IV)

The microscopy samples used in Study IV were prepared in laboratory conditions using previously acquired, anonymized stool and urine samples. The stool samples had been concentrated with ethyl acetate and formalin-fixed prior to storage. In total, three separate stool samples, microscopically confirmed positive for parasites were acquired. Of these, one sample contained hookworm eggs, one *A. lumbricoides* and one containing both *T. trichiura*

and *A. lumbricoides.* Furthermore, we acquired an unfiltered urine sample containing *S. haematobium*. These samples originated from sample series collected for educational and quality assurance purposes, and were not been specifically collected for this study. Faecal samples were obtained from the Department of Microbiology and Immunology, University of Helsinki, Finland and the urine sample from the diagnostic parasitology laboratory of the Swedish Institute for Communicable Diseases Control ('Panel för Cystor & Maskägg', SMI, Solna, Sweden). Using the stool samples, microscopy glass slides were prepared using routine iodine staining (equal part sample and staining solution). Fixation on the glass slides was performed using a solution containing 40% acrylamine, phosphate buffer solution (PBS), tetraacetylethylenediamine (TEMED) and ammonium persulfate. The corresponding mixture of fixation solution and faecal sample was placed on glass slides and covered with cover slips. For the urine samples, equal parts urine sample and acrylamide solution were correspondingly measured and placed on microscopy glasses and covered with cover slips. To prevent drying, edges of the cover glasses were treated with a mounting media solution. Using this method, 30 fixated stool samples and 10 urine samples were prepared and microscopically examined for presence of parasites. Slides negative for parasites were excluded, after which a total of nine glass slides showing *A. lumbricoides,* two showing *T. trichiura*, five showing hookworm eggs and four urine samples showing *S. haematobium* remained for further analysis.

As this article reported a study performed on samples archived for educational purposes, and thus containing no personal identifiers or clinical records, written informed consent was not required according to the Ministry of Social Affairs and Health, Finland Act on the Medical Use of Human Organs, Tissues and Cells (Amendments up to 277/2013 included). Ethical approval for this study was approved by the study was approved by the Coordinating Ethical Committee of Surgery of the Hospital District of Helsinki and Uusimaa (DNo. HUS/1655/2016).

4.2 Digitization of samples

For the digitization, i.e. scanning, of microscopy samples in the studies we used in total three separate slide scanning methods. In study I, II and IV the samples were digitized using two separate devices; a prototype POC miniature digital microscope and a laboratory-grade

high-end whole slide scanner (for benchmarking and reference purposes). In study III, the samples were digitized using a commercially available portable digital slide scanning platform. The technical specifications of the devices are described in more detail below.

The prototype POC miniature digital microscope scanner used for sample digitization in Studies I, II and IV is a portable, lightweight and cloud-connected digital microscope scanner prototype ("MoMic"), developed by the Institute for Molecular Medicine Finland (FIMM) at University of Helsinki (Figure 7) in collaboration with University of Oulu and Karolinska Institutet. The device is built using inexpensive, mass-produced plastic optoelectronic components, typically used in consumer electronic products, such as smartphone camera systems. By using this type of components, the total material costs are reduced by multiple orders of magnitude (approximately 500 - 1.000 EUR), compared to conventional slide scanners retail prices (approximately 30.000 - 200.000 EUR). The system utilizes a white light-emitting diode (LED) as the illumination source for brightfield imaging and supports transmitted light fluorescence imaging using a retractable ultraviolet (UV) LED source with adjacent filters. For the imaging sensor, a 13-megapixel complementary metal oxide semiconductor (CMOS) sensor is used with a total image resolution of 4208 x 3120 pixels. The reversed, plastic 1/3.2" lens has a field of view (FOV) of approximately 0.84 x 0.62 mm^2, resulting in a pixel size of approximately 0.22 µm x 0.22 µm with a spatial resolution of 0.9 µm as measured using a standardized USAF resolution test chart (Figure 8), and a magnification of approximately 5.5X. An external motor unit is used for sample adjustment when digitizing samples measuring multiple FOVs. The device is connected to a laptop computer through a universal serial bus (USB) connector, and controlled using a custom software. The software features a live-view from the device camera and controls to adjust sample position and scanning settings. Acquired images are saved on the local storage of the computer or uploaded to an image processing and management platform (WebMicroscope, Fimmic Oy, Helsinki, Finland), running on a cloud server at the university campus. When scanning multiple FOVs, corresponding whole slide images (WSIs) are constructed from the individually captured images using the commercially available software suite Image Composite Editor (Microsoft Computational Photography Research Group, Microsoft Inc., Redmond, WA). Generated WSIs are saved in the Tagged Image File Format (TIFF) and compressed to a wavelet file format prior to uploading (Enhanced Compressed Wavelet, Hexagon Geospatial, Madison, AL, USA) with a target compression ratio of 1:9. This amount of compression preserves sufficient spatial

image quality to not alter image analysis results significantly, as shown in previous work (Konsti et al., 2012). Remote access to samples at the cloud server can be established using a web browser secured with the secure sockets layer (SSL) encryption technology.

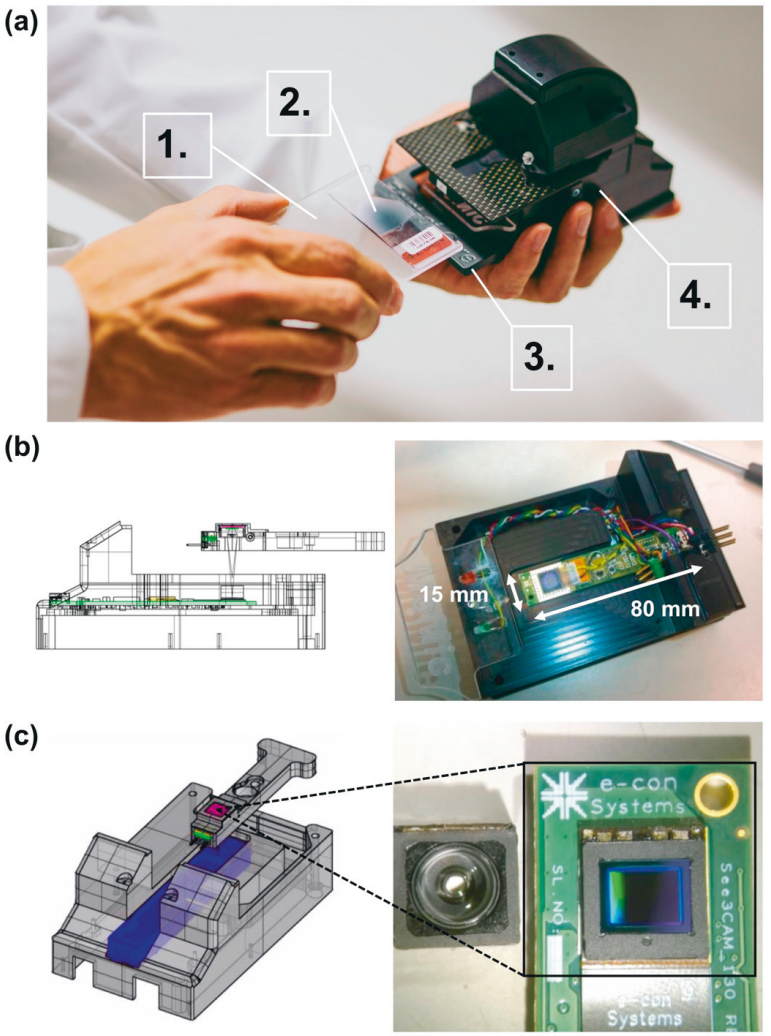


Figure 7. FIMM 'MoMic' mobile microscope prototype. Image **(a)** showing the point-of-care microscope prototype with sample holder **(1)**, glass slide **(2)**, focus adjustment lever **(3)** and main microscope unit **(4)**. Image **(b)** showing schematic view of device internals, with size of main printed circuit board, and disassembled camera module **(c)** with the miniaturized lens and imaging sensor used to capture digital images. *Partly modified from from PLOS (Holmström et al., 2015).*

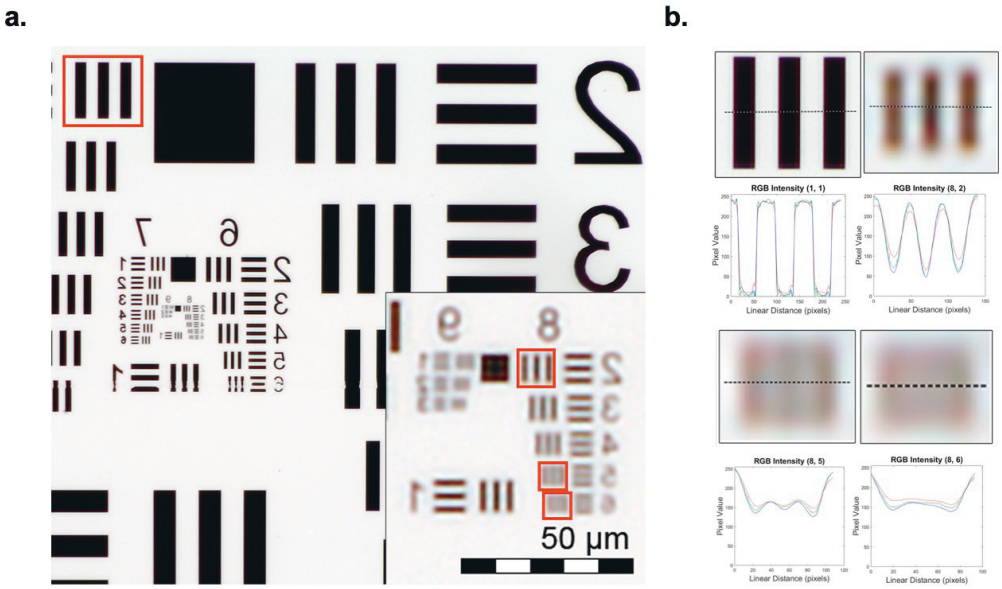


Figure 8. USAF Chart, captured with early Momic prototype. **(a)** US Air Force 1951 standardized three-bar resolution test chart, with pixel intensity profiles **(b)**, as measured over smallest set of resolvable bars (group 8; element 5) and group 8; element 6 (unresolvable). *Adapted with permission from PLOS (Holmström et al., 2015).*

The reference high-end slide scanner used in Studies I, II and IV is a conventional laboratory-grade, high-end whole slide scanner (Pannoramic 250 FLASH, 3DHistech Ltd., Budapest, Hungary). Scanning was performed using a 20x objective with a numerical aperture (NA) of 0.8, using a three-CCD (chard-coupled device) digital camera with a 1.0 adapter, rendering a spatial resolution of 0.22 µm/pixel. Captured digital slides were saved locally or compressed to ECW format and uploaded to the WSI management server using the configurations described above.

In Study III, samples were digitized using a small-sized, commercially available portable digital microscope scanner (Grundium Ocus, Grundium Oy, Tampere, Finland) (Figure 9). The device uses an 18-megapixel image sensor with a 20x objective (NA 0.40) and a pixel size of 0.48 µm. The scanner is connected to a computer over a WLAN connection and controlled through a browser interface. Coarse focus for the scanner can be manually adjusted and fine focus adjusted using the built-in autofocus routine. Images were saved in the Tagged Image File Format (TIFF) on a local hard drive, and converted to ECW format

with a 1:16 compression ratio, before uploading to an image management platform (Aiforia Cloud, Aiforia Technologies, Helsinki, Finland).

Figure 9. Point of care digital laboratory in Kenya. Image showing computer with cloud-based slide management platform **(1)**, POC digital slide scanner **(2)**, mobile network router **(3)** and glass slides (Pap smears) **(4)**.

4.3 Digital image analysis methods

For the digital image analysis, we used in total three separate computer digital image analysis algorithms. Two of these utilized machine learning with deep convolutional neural networks (CNNs), and one method was based on a more conventional computer vision approach with colour deconvolution and thresholding to separate stains and objects from the background.

In Study I, the quantitative image analysis of the digital samples was performed using the image-analysis library ImmunoRatio2 (Jilab Inc., Tampere, Finland). The software uses colour deconvolution to separate individual stains and nuclear thresholding, particle segmentation and filtering techniques to distinguish cellular nuclei in the images

(Tuominen et al., 2010, Ruifrok, Johnston, 2001). In this way the total amount of positive and negative nuclei in the images can be calculated to yield an overall value for percentage of IHC positive cells (ER positive cells in this application). For the calibration of the software, a training series consisting of 10 digital samples was selected and used to configure the software (i.e. adjusting parameters such as colour thresholds, pixel size and nuclei size). By adjusting threshold values for the algorithms (colour thresholds for separation of DAB and haematoxylin stains and average nuclei sizes in proportion to pixel size of images, based on analysis performance in the training set) the algorithm was calibrated to quantify individual nuclei in the digitized samples and classify them as ER positive or negative. When the best-performing configuration was achieved on the training series, analysis of the test series (170 samples) was performed without human supervision.

In Study III, we trained a deep CNN model, running on a cloud server, to detect squamous cervical-cell atypia in digitized Pap smears. For this, we utilized a commercially available machine learning and image analysis platform (Aiforia Cloud, Aiforia Technologies, Helsinki, Finland). Training of the algorithm was performed using a subset of the samples from the study, representing 50 % of the target number of samples in the study plan (n = 350). The deep learning system (DLS) was trained to detect regions of low- and high-grade squamous cell atypia (defined as LSIL, or higher) in the digitized samples (Figure 10). The area of the digital Pap smear whole slides measured approximately 100,000 x 50,000 pixels. For the training of the DLS, representative, separate regions (n = 16,899) with cross-sections of ~25-100 µm were selected visually from the training slides. The regions represented areas of both normal cervical morphology and cervical-cell atypia of different degrees. The DLS was trained using 28 000 training epochs (iterations), using a pre-determined feature size (field-of-view) of 30 µm. To maximize the generalizability of the model, the training data was augmented using various image perturbations; variation in scale (± 10%), aspect ratio (± 10%), shear distortion (± 10%), luminance (± 10%), contrast (± 10%), white balance (± 10%) and variation in image compression quality (40–60%).

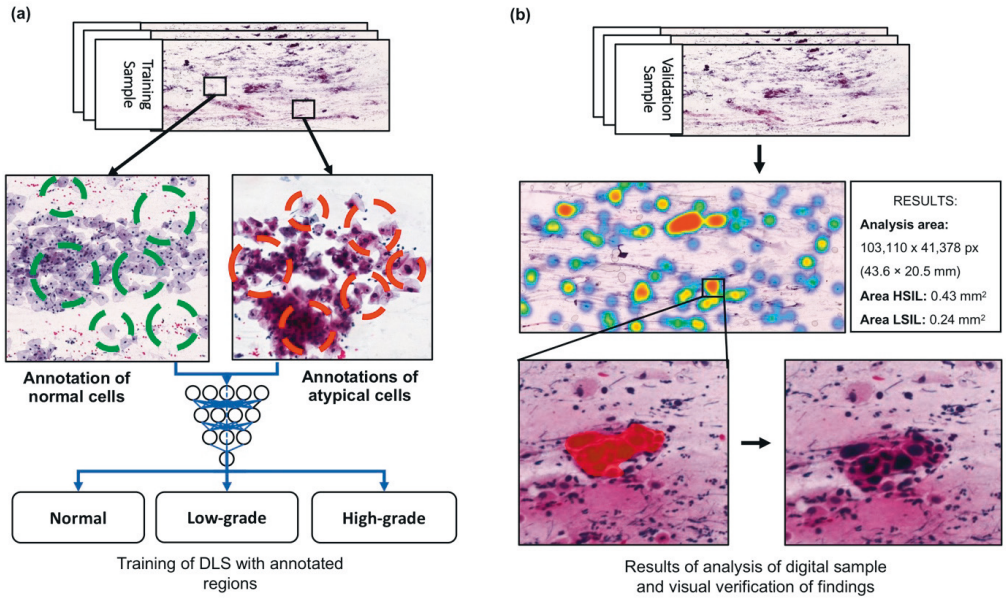


Figure 10. Schematic view of training and analysis using the deep-learning system (DLS). Image showing manual annotation of regions of normal and atypical cells **(a)**, which are used to train the DLS to detect and classify atypical findings and DLS-analysis of digital sample **(b)** with results in numerical and visualized with heatmap colour overlays for manual verification.

In Study IV, we also trained a supervised deep CNN-based algorithm to analyse the samples. For the training of the algorithm, a subset of the digitized images with visible parasites were used as training data. From the total number of images captures with the scanner (n = 7385), we used 218 (3 %) images for this purpose. The development and training of the deep learning model was performed using a commercially available image management and analysis platform (WebMicroscope, Fimmic, Helsinki, Finland) based on the manual labelling of digital samples in the training series. The pipeline for image analysis, i.e. detection and classification of parasites, involved two sequential algorithms. The first algorithm was trained using labelled training data were all visible parasites in the images had been manually annotated on an object-level, regardless of species. Based on these predefined objects of interest (location in image and size), the algorithm was trained to

detect all visible parasites in the samples, but not yet classify them according to species. The detected objects of interest by the first algorithm were fed forward to a second classifier, trained to perform the classification of detected parasites into likely species. The second classifier layer analysed exclusively the objects detected by the first algorithm (200 x 200 pixels) to determine the most likely species. If no suitable match was detected, the finding was classified as "other" (e.g. artefact). Training of this classifier was also performed using manually labelled data for the different parasites. Using this two-phase method, the findings in the analysed samples were exported as a report for manual confirmation, arranged with the findings most likely to represent parasites first (Figure 11).

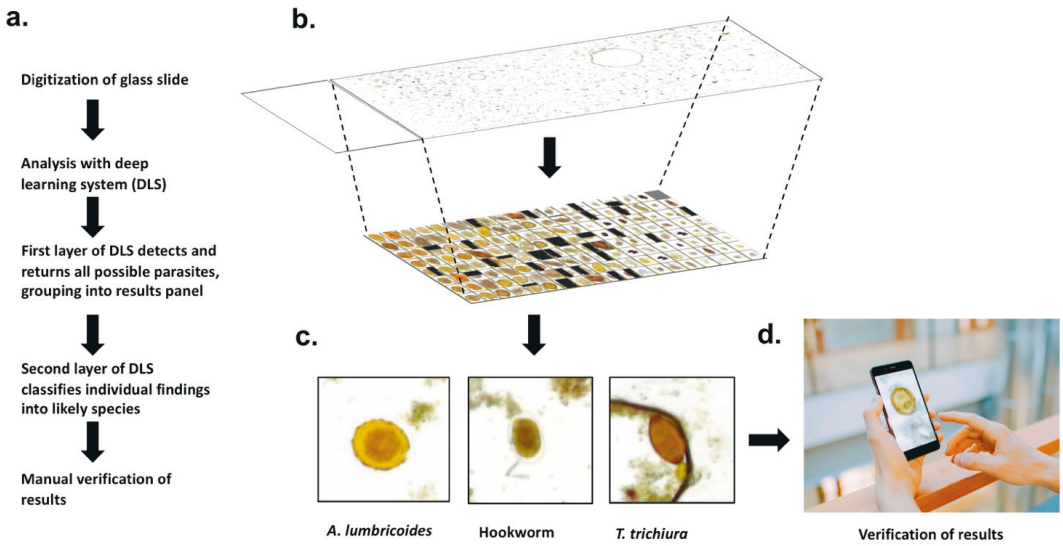


Figure 11. Workflow of digital analysis of sample with deep learning system (DLS) to detect and classify Neglected Tropical Parasites. Image showing sample analysis pipeline **(a)**, analysis of whole slide-image and grouping of all potential parasites into one image panel **(b)**, classification of parasites with second layer of DLS **(c)** and exported results for visual verification **(d)**.

4.4 Statistical analysis

Statistical analyses for the studies were performed using the general-purpose statistical software packages SPSS 22.0.0 for Windows (SPSS Inc., Chicago, USA), and Stata 15.1 for Mac (Stata Corp., College Station, TX, USA). Kappa statistics were used to assess inter-rater agreements with values 0.01–0.20 considered as slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 significant and 0.81–1.00 as almost perfect agreement (Landis, Koch, 1977), using linear weights when applicable. Correlation coefficients were calculated with the Pearson product-moment correlation method and Bland-Altman plots were used to visualize inter-rater agreements between observers. Diagnostic sensitivity was calculated as the percentage of true positives (TP) divided by TP and false negatives (FN). Positive predictive value (PPV) on an object level was calculated as the percentage of true positives divided by true positives and false positives (FP). Statistical-power calculations were performed with a sample-size formula (Buderer, 1996), in Study III assuming a disease prevalence (P_r) of 8% (± 2%) in the study population (based on previous literature), and $\alpha = 0.05$ (and correspondingly $Z_{1-\alpha/2} = 1.96$), and a precision parameter (ε) of 0.10, to determine whether sensitivity (S_N) and specificity (S_P) were comparable to the ground truth.

$$n_{Sensitivity} = \frac{Z_{1-\alpha/2}^2 S_N(1-S_N)}{\varepsilon^2 \times P_r}$$

$$n_{Specificity} = \frac{Z_{1-\alpha/2}^2 S_p(1-S_p)}{\varepsilon^2 \times (1-P_r)}$$

All statistical tests were two-sided unless otherwise stated. Unless otherwise mentioned, the level of statistical significance was 0.05 for all values displayed. Assessment of the diagnostic performance of the DLS was performed by calculating the area under the curve (AUC) of the receiver operating characteristics (ROC) curves, as plotted with the true-positive rate (TPR; sensitivity) versus the false-positive rate (FPR; 1 – specificity) for different thresholds of slide-level positivity for the algorithms. Statistical estimates of diagnostic accuracy were reported with 95% confidence intervals (95% CIs).

5 RESULTS

5.1 Breast cancer histopathology with low-cost, point-of-care digital microscopy (Study I & II)

In Studies I and II we evaluated the histopathological assessment of breast cancer ER-receptor status and detection of metastases in sentinel lymph node frozen sections, using samples digitized with a POC prototype miniature microscope. The samples were analysed both manually and digitally, and results were compared to the visual analysis of samples and to digital analysis of samples scanned with a high-end laboratory-grade whole-slide scanner as the reference (Table 2).

First, a computer vision software was configured and applied to analyse the digital samples, scanned with both devices. The samples were analysed to determine overall ER positivity by quantifying the percentage of cells expressing ER. The ground truth to which image analysis results were compared was the conventional, visual assessment of ER positivity in the digital samples, as classified according to cut-off values from clinical guidelines (Hammond et al., 2010); i.e. strongly ER-positive (> 10% ER-positive cells), weakly ER-positive (≥ 1% ER-positive cells) or ER-negative (< 1% ER positive cells). In total, after exclusion of 10 samples used for the calibration of the algorithm, 170 samples were analysed. In Study II, the same devices were used to digitize intraoperative samples from breast cancer surgeries, and the digital samples from both the high-end and low-cost devices reviewed remotely by two independent pathologists to detect metastases. After two inadequate samples were excluded, 152 slides remained for analysis. Results were compared between the devices, with the conventional pathologist light-microscopy diagnosis of the glass slides as the reference.

Table 2. Samples used in breast cancer studies (Study I and II) with results from analysis with the different methods (visual analysis and digital analysis of samples scanned with the reference high-end scanner and the low-cost point-of-care device).

Breast cancer core biopsies (Study I)

Level of ER-positivity	Visual sample analysis, *n* (%)	Analysis of high-end scanner samples, *n* (%)	Analysis of point-of-care microscope samples, *n* (%)
Negative (< 1%)	33 (19.4)	19 (11.2)	17 (10.0)
Weakly ER-positive (< 10%)	18 (10.6)	21 (12.4)	25 (14.7)
Strongly ER-positive (> 10%)	119 (70.0)	130 (76.5)	128 (75.3)

Breast cancer surgery frozen sections (Study II)

Frozen section metastasis status	Visual sample analysis, *n* (%)	Analysis of high-end scanner samples, *n* (%)*	Analysis of point-of-care microscope samples, *n* (%)*
No metastasis present	103 (67.8)	106 (69.7)	107.5 (70.7)
Metastasis present	49 (32.2)	46 (30.3)	44.5 (29.3)

Number of samples shown with corresponding percentage of total number of samples in studies (n = 170 in Study I and n = 152 in Study II)

*Mean values from pathologists reviewing the samples

5.1.1 Digital image analysis compared to conventional sample analysis for analysis of breast cancer samples

In total, 170 breast cancer tissue samples were analysed for ER-positivity. We compared the detected ER-positivity, measured by digital image analysis, to the visual analysis of the samples. The reference visual results of the samples yielded 33 (19%) samples as ER-negative, 18 (11%) as weakly ER-positive and 119 (70%) as strongly ER-positive (Table 2). When comparing these results to the image analysis results (Figure 11) of the digital samples from the miniature scanner, a strong correlation was observed ($r = 0.94$, $p < 0.001$) with a substantial level of agreement ($\kappa = 0.71$; 95% CI 0.61 - 0.80). Similarly, when comparing results from the visual sample analysis to the digital image analysis of samples from the high-end scanner, comparable levels of correlation ($r = 0.93$, < 0.001) and agreement ($\kappa = 0.69$; 95% CI 0.60 - 0.78) were observed (Table 2). Overall, 16 (9%) discrepant samples, compared to the ground truth, were observed in the digital image analysis of samples from the miniature scanner, and 14 (8%) in the reference slide scanner samples. All of these represented false-positive (FP) samples, i.e. classified as positive by the image

analysis and negative by manual analysis. Notably, no false-negative (FN) samples were observed in the results from either device.

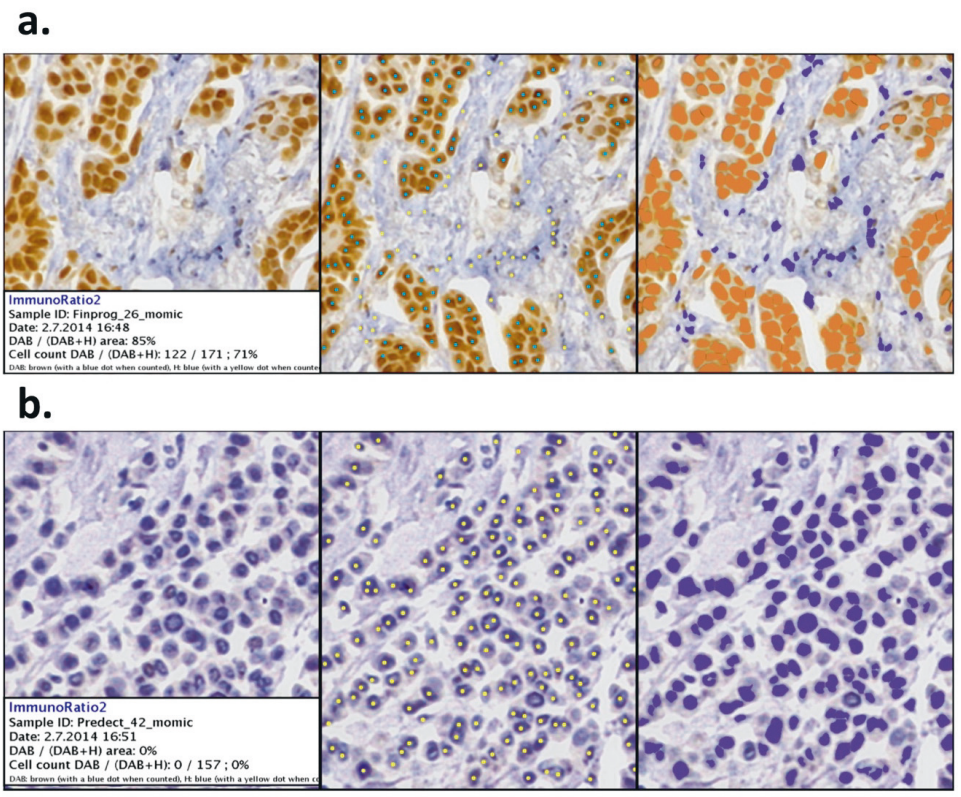


Figure 11. ER-positive and ER-negative breast cancer samples. Image showing ER-positive **(a)**, and ER-negative **(b)** breast cancer samples, digitized with the low-cost microscope, analysed with the ImmunoRatio algorithm with detected ER-positive (blue dots) and ER-negative (yellow dots) cells and numerical results from image analysis.

5.1.2 Analysis of breast cancer tissue samples with a high-end, laboratory-grade scanner and a miniaturized, low-cost scanner

Next, we compared the results from the digital image analysis of samples scanned with the high-end and the low-cost devices. Overall, the digital image analysis results correlated strongly when comparing numerical results from both devices in terms of detected ER-positivity ($r = 0.98$, $p < 0.001$) (Figure 12). We calculated a substantial level of inter-rater

agreement between the results from both devices, as measured using unweighted kappa statistics (κ = 0.84) and no statistically significant differences were observed in inter-rater agreement with both devices when compared to the ground truth. The principal challenge for the image analysis was the analysis of weakly ER-positive samples, but the overall rate of discrepancies was generally low and similar for both devices. Notably, no false-negative (FN) samples were observed with either device (Table 3).

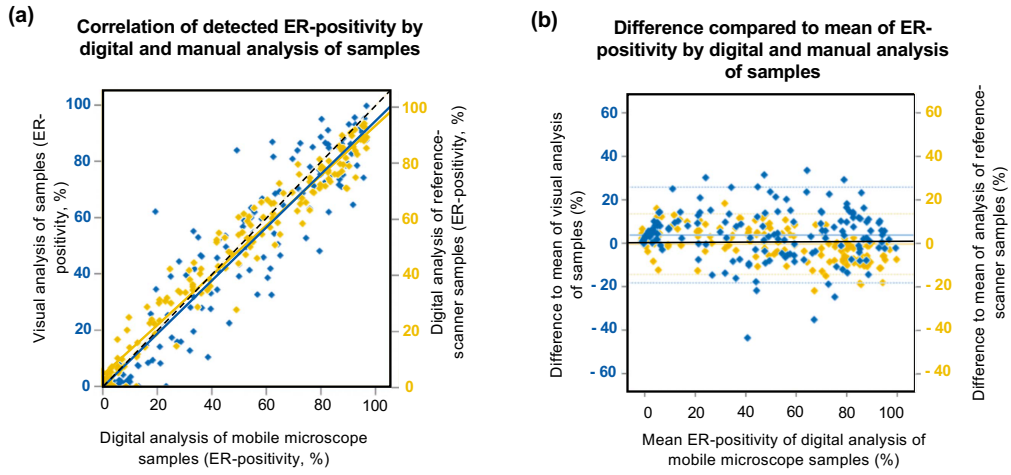


Figure 12. Comparison of results between digital image analysis and visual sample assessment. Image showing scatter plots of detected estrogen receptor (ER) -positivity by visual and digital analysis of samples **(a)**, and Bland-Altman plots illustrating the inter-rater agreement of the different methods as difference compared to mean of the results of detected ER-positivity **(b)**.

Table 3. Inter-rater agreement and diagnostic performance for analysis of samples from the POC miniature scanner and the high-end slide scanner, compared with the ground truth assessment of samples.

Diagnostic comparison	Inter-rater agreement, κ (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	False positive, *n* (%)	False negative, *n* (%)
Assessment of ER-positivity by digital image analysis (POC scanner)*	0.71 (0.61 - 0.80)	100.0 (97.34 - 100.0)	51.5 (33.54 - 69.2)	16 (9%)	0 (0%)
Assessment of ER-positivity by digital image analysis (high-end scanner)*	0.69 (0.60 - 0.78)	100.0 (97.34 - 100.0)	57.6 (39.22 - 74.5)	14 (8%)	0 (0%)
Visual remote detection of metastases in samples (POC scanner)**	0.91 (0.85 - 0.98)	89.8 (77.8 - 96.6)	99.0 (94.7 - 100.0)	1 (1%)	5 (3%)
Visual remote detection of metastases in samples (high-end scanner)**	0.95 (0.90 - 1.00)	93.9 (83.1 - 98.7)	99.0 (94.7 - 100.0)	1 (1%)	3 (2%)

Kappa values (unweighted), sensitivity and specificity shown with associated 95% confidence intervals (95% CI). Numbers of false-positive and false-negative slides shown with the corresponding percentage of total slides analysed (n = 170 in Study I and n = 152 in Study II)

*Sensitivity and specificity calculated for detection of overall ER-positive and negative samples

**Results calculated as mean values from the pathologists

Similarly, when comparing results between devices for the detection of metastases in the digital samples scanned with both devices, we observed a high level of agreement in results from both the same devices. Overall, 152 samples were reviewed by two independent pathologists for the detection of breast cancer metastases. Here, overall sensitivity (89.9% and 93.9%, respectively) and specificity (99.0% and 99.0%) were similar for interpretation of slides from both devices. When calculating inter-rater agreement in results from the pathologists, the remote interpretation of slides yielded very similar results for both the miniature microscope ($\kappa = 0.91$) and the reference high-end scanner ($\kappa = 0.95$), compared to the conventional light microscopy diagnosis and no statistically significant differences were observed between the devices (Figure 13). The overall number of discrepant slides was low for both devices. The percentage of FN slides in the analysis of the miniature scanner slides were 2% and 4%; compared to 0% and 2% with the reference slide scanner. The percentage of FP samples were 0% and 1 % with the miniature microscope slides, and 0% and 1 % with the reference slide scanner (Table 3). A majority of the incorrectly classified slides on a slide level were toluidine blue slides with micrometastases, which were correctly diagnosed in corresponding IHC-stained sections when available.

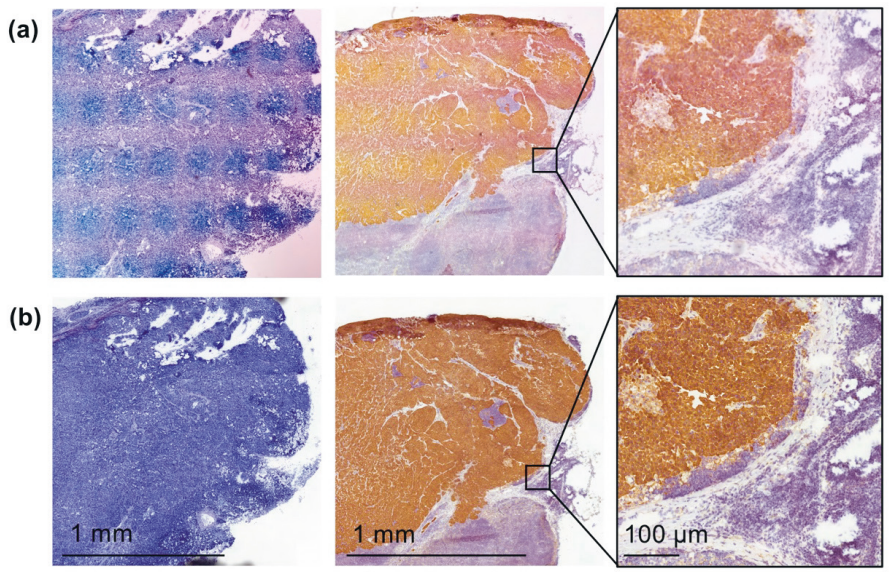


Figure 13. Digitized lymph node frozen sections (FS). Stained sample (toluidine blue and anti-CK) with visible breast cancer metastasis. Row **(a)** showing samples scanned with the miniature microscope scanner, and row **(b)** showing corresponding samples scanned with the high-end reference slide scanner.

In conclusion, Studies I and II demonstrate that the image quality achievable with a portable, miniaturized and inexpensive digital microscope scanner is sufficient for certain routine histopathological applications in breast cancer diagnostics. Specifically, the results demonstrate that analysis of the digitized samples for detection of breast cancer metastases in lymph node frozen sections is feasible and that determination of tumor characteristics (expression of hormone receptors in immunostained samples) is possible both visually and using digital image analysis algorithms, with results similar to conventional methods.

5.2 Cloud-based deep learning for detection of cervical-cell atypia in digitized Pap smears (Study III)

A POC digital slide scanner was deployed at a rural clinic in Kenya and used to digitize a total of 720 Pap smears on-site. Using the local data networks, the samples were uploaded to a cloud server and analysed with a deep learning system (DLS), trained to detect atypical lesions (low- and high-grade squamous intraepithelial lesions). Results were compared to the expert visual assessment of the digital slides and to the assessment of the physical glass slides by the local pathologist.

5.2.1 Detection of cervical-cell atypia with the DLS compared to the expert visual assessment of digital samples

After exclusion of 29 (7%) ineligible samples, 361 samples remained in the validation series. The expert visual assessment of the digital samples was performed remotely by a cytotechnologist and pathologist, both experienced in cervical cytology microscopy. The cytotechnologist initially screened all digital samples, and all slides with significant cervical cellular atypia (LSIL, or higher) were confirmed by the pathologist. Furthermore, according to generally accepted guidelines, 10% of slides classified as normal by the cytotechnologist were randomly selected and submitted for re-analysis by the pathologist. In this visual assessment of samples, 19 (5%) of slides presented with low-grade and 28 (8%) with high-grade atypia and 314 (87%) were classified as negative for significant squamous cell atypia. With these results as a reference, the DLS achieved an area under the receiver operating

characteristic curve (AUC) of 0.94 with a sensitivity of 95.7% (95% CI 85.5–99.5%) and a specificity of 84.7% (95% CI 80.2–88.5%) (Figure 20). For the detection of slides with high-grade lesions, the AUC was 0.93, the sensitivity 85.7% (95% CI 67.3–96.0%) and specificity 86.0% (95% CI 81.8–89.5%). The AUC for the detection of low-grade slides was 0.86, with a sensitivity of 84.2% (95% CI 60.4–96.6%) and a specificity of 86.0% (95% CI 81.8–89.5%) (Table 3). Compared to the expert assessments of the digital slides, two slides with low-grade atypia were classified as negative by the DLS (<1%), but no high-grade slides were falsely classified as negative by the DLS, although four high-grade slides (1%) were classified as low-grade by the DLS (Table 3). In these results, the negative predictive value (NPV) for the DLS was high for general atypia (266/268 = 99.3%; 95% CI 97.3–99.9%), low-grade atypia (294/297 = 99.0%; 95% CI 97.1–99.8%) and high-grade atypia (328/332 = 98.5%; 95% CI 96.9–99.7%).

5.2.2 Detection of cervical-cell atypia with the DLS compared to expert visual assessment of physical samples

We compared the performance of the DLS for detection of cervical-cell atypia to the assessment of the physical slides, performed by a local pathologist. For this, we obtained the cytological reports from the glass slides from the local pathology laboratory. Of the slides in the validation series, the local pathologist classified 342 (95%) as negative for significant squamous-cell atypia, 14 (4%) as positive for low-grade atypia and five (1%) as positive for high-grade atypia. With these results as the reference, the DLS achieved a sensitivity for general atypia of 100% (95% CI 82.4–100%) and for high-grade atypia of 100% (95% CI 47.8–100%); with corresponding levels of specificity of 78.4% (95% CI 73.6–82.4%) and 93.3% (95% CI 90.1–95.6%), respectively (Table 4). We calculated a lower sensitivity for low-grade atypia (21.4%; 95% CI 4.7–50.8), as 11 of 14 slides classified as low-grade atypia in the report from the local pathologist were classified as high-grade by the DLS. We observed a high NPV for general atypia (266/266 = 100%; 95% CI 98.6–100.0%), for high-grade atypia (332/332 = 100%; 95% CI 98.9–100.0%) and low-grade atypia (286/297 = 96.3 %; 95% CI 93.5–98.1%). Overall, the DLS achieved high AUCs for detection of general atypia (0.96), high-grade atypia (0.94) and low-grade atypia (0.94) (Figure 14). Compared to the physical-slide cytodiagnosis, no atypical slides were falsely classified as negative by the DLS.

In conclusion, Study III demonstrates how a miniaturized POC digital microscopy scanner can be implemented at a rural clinic in a region with high cervical cancer prevalence and low screening coverage, and used to digitize samples for automatized deep learning-based analysis to detect atypical lesions. The results showed high agreement to visual assessment of samples by pathologists and high negative predictive values, suggesting that this technology could be utilized for screening purposes.

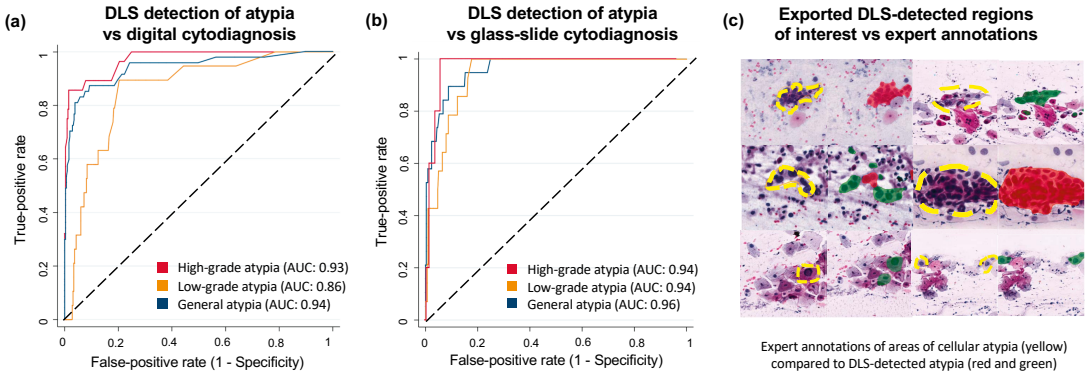


Figure 14. Results from analysis of Pap smears with the deep learning system (DLS). Results from the DLS-based analysis, compared to the expert sample assessment of digital samples **(a)**, and physical glass slides **(b)**. Image **(c)** showing regions marked by expert sample assessment and corresponding areas as detected by the DLS.

Table 4. Results from analysis of digitized Pap smears with the deep learning-system with the expert assessment of samples as reference.

Diagnostic comparison	Sensitivity, % (95% CI)	Specificity, % (95% CI)	False negative, *n* (%)	False positive, *n* (%)	True negative, *n* (%)	True positive, *n* (%)
Expert assessment of digital slides						
Atypia in general	95.7 (85.5-99.5)	84.7 (80.2-88.5)	2 (0.6)	48 (13.3)	266 (73.7)	45 (12.5)
High-grade atypia	85.7 (67.3-96.0)*	98.5 (96.5-99.5)	4 (1.1)*	5 (1.4)	328 (90.9)	24 (6.6)
Low-grade atypia	84.2 (60.4-96.6)	86.0 (81.8-89.5)	3 (0.8)	48 (13.3)	294 (81.4)	16 (4.4)
Expert assessment of glass slides						
Atypia in general	100 (82.4-100)	78.4 (73.6-82.6)	0 (0)	74 (20.5)	268 (74.2)	19 (5.3)
High-grade atypia	100 (47.8-100)	93.3 (90.1-95.6)	0 (0)	24 (6.6)	332 (92.0)	5 (1.4)
Low-grade atypia	21.4 (4.7-50.8)**	69.2 (78.0-86.3)	11 (3.0)**	61 (16.9)	286 (79.2)	3 (0.8)

Calculated sensitivity and specificity of the deep-larning system (DLS) are shown with the associated 95% confidence intervals.
Numbers of false-negative, false-positive, true-negative and true-positive assessments are shown with the corresponding percentage of the total number validation slides (*n* = 361).

*Findings in sides classified by experts as high-grade atypia were classified as low-grade atypia by the DLS.
**Findings in slides classified by experts as low-grade atypia were classified as high-grade atypia by the DLS.

5.3 Diagnostics of Neglected Tropical Diseases with deep learning-based image analysis and point-of-care digital microscopy

A model based on deep convolutional neural networks was trained to analyse digital samples and both detect and classify tropical parasites. Samples were digitized using a low-cost, slide-scanning platform, and results compared to visual assessment of samples.

5.3.1 Visual assessment of samples and establishment of study ground truth

The parasitological samples were digitized using both the POC and high-end scanners and corresponding digital slides (whole slide images) visually examined on a computer monitor to confirm that parasites could be visualized in the scanned images. By visual assessment spatial resolution was determined to be sufficient for both devices to clearly identify parasites and reliably determine species for the parasites studied in the digital images (Figure 15). To establish the study ground truth, the digital samples were reviewed by two independent researchers and location and species of all parasites detected recorded. The corresponding annotations were both observers agreed on parasites were used as the ground truth for the analysis.

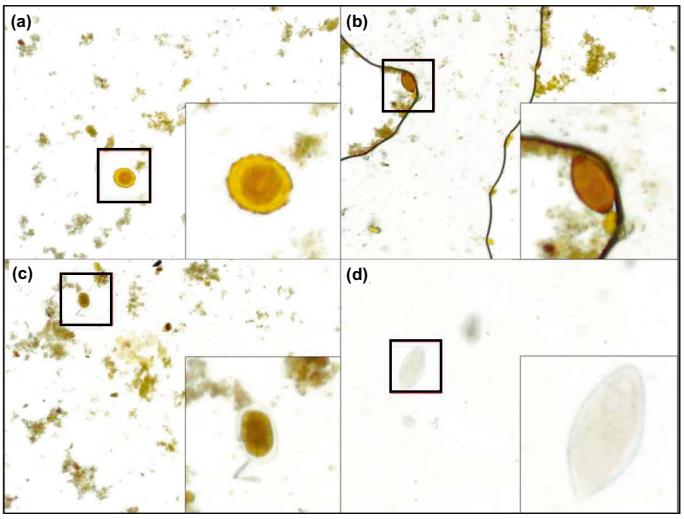


Figure 15. Digitized image, captured with the point-of-care microscope prototype, showing visible parasites. Enlarged areas showing visible parasites; *A. lumbricoides* **(a)**, *T. trichiura* **(b)**, hookworm **(c)**, *S. haematobium* **(d)**. *Image adapted with permission from Taylor & Francis Group (2017).*

5.3.2 Deep learning-based detection of parasites in digital samples

For analysis by the deep learning system (DLS) all digital images captured with the miniature microscope were pooled, resulting in a total of 7385 images. By visual analysis, 410 of these images were classified as positive (i.e. containing observable parasites) with a total of 434 manually labelled helminth eggs. Of these, 390 were labelled as *A. lumbricoides,* 12 as *T. trichiura* and 32 as hookworm eggs. In total, 50% of samples were used to train the DLS, and 50% for verification of results. On an object level, the sensitivity for detection of *A. lumbricoides* in the test series was 100%, i.e. all visual parasites in the scanned samples were correctly detected by the software. The algorithm detected 13 false positive (FP) *A. lumbricoides* parasites, and correspondingly no false negative (FN) samples for an overall positive predictive value (PPV) of 93.7% on an object level. For *T. trichiura*, diagnostic sensitivity was 83.3% on an object level, no FP cases were observed and one FN case was detected, resulting in a PPV of 100% on an object level. Sensitivity for detection of hookworms in the digital samples was 93.8%, with 7 FP cases and one FN, yielding a PPV of 96.9% on an object level. Following sample analysis, results (detected objects of interest) were automatically exported and presented as a single panel, in order from most likely detected parasites to least likely (Figure 16).

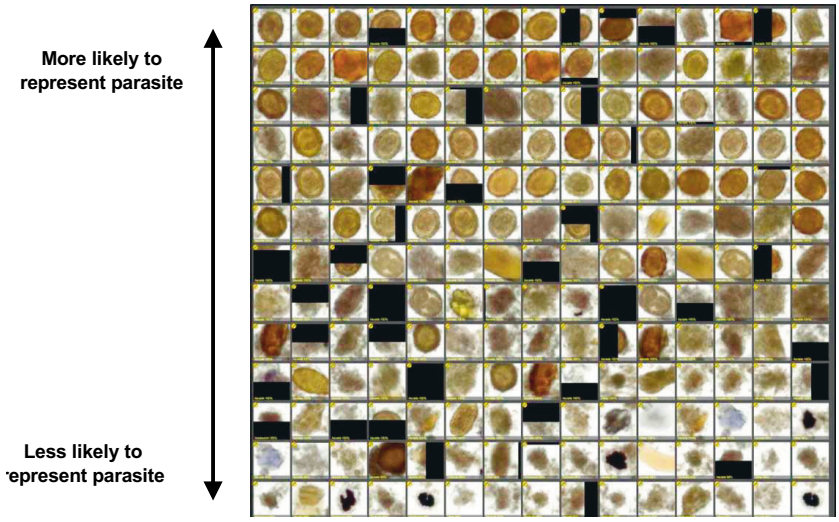


Figure 16. Panel showing detected objects of interest from image analysis of fixated stool sample, showing detected *A. lumbricoides* parasite ova; arranged in descending order according to likelihood of representing parasite. Tiles with black boxes are objects detected at edges of the sample. *Image adapted with permission from Taylor & Francis Group (2017).*

In conclusion, the findings from Study IV demonstrates that the imaging performance of an inexpensive, portable microscopy scanner, suitable for rural POC usage, is sufficient to detect the most common neglected tropical diseases. Furthermore, by applying an automatized deep-learning based algorithm, the sample analysis process can be facilitated by automatically identifying parasites with relatively high levels of sensitivity (83 – 100%, depending on species), classifying the findings according to species and presenting the most significant findings arranged into an image panel for rapid manual verification.

6 DISCUSSION

Light microscopy has remained a relatively unchanged diagnostic technique for over a century. Driven by technological advancements, the fundamentals of this common diagnostic procedure are undergoing a paradigm shift towards digital methods (Djuric et al., 2017). Technologies such as digitization of samples for automatized digital image analysis with machine learning-based models present opportunities to facilitate the diagnostic process by providing rapid, accurate and reproducible results for a variety of diagnostic applications. As access to diagnostics for many common and treatable conditions is severely limited in many areas, there is a well-recognized need for improved diagnostic techniques (World Health Organisation, 2017). A barrier to implementing digital technologies has long been the requirement for expensive and large-sized equipment, which is not suitable for usage in rural areas. By utilizing advancements in components from consumer electronics, inexpensive and portable optical imaging devices (digital microscopes) have since been developed and shown to have potential as platforms for digital diagnostics at the front line. Similarly to the rapid expansion of mobile phone networks which has completely leapfrogged conventional land lines, novel diagnostic technologies here have the potential to be implemented more efficiently and rapidly (Sinha, Barry, 2011). While adaption of novel technologies in high-resource settings is often associated with increased costs (MIT Technology Review, 2013), in lower-resource settings there is an opportunity to define standards of diagnostics that can be implemented efficiently and inexpensively (Richards-Kortum, Oden, 2013). Improved access to diagnostics has significant possible health impacts by e.g. directly saving lives (Girosi et al., 2006) and reducing overtreatment, and even minute improvements (by e.g. 1%) in diagnostic accuracy can translate into sizeable positive effects (Lim et al., 2006). Moreover, it is likely that the implementation of these technologies is feasible without large economical investments (Fleming et al., 2017).

The aim of this thesis was to study how POC digital microscopy, supported by digital image analysis techniques can be used for diagnostics of various diseases, with a focus on potential applications in low-resource areas. Previous studies have suggested a number of devices, mainly utilizing smart phone components, as potentially usable systems for digitization of microscopy samples in field settings (Saeed, Jabbar, 2017). To date, these have been evaluated mainly for diagnostics of infectious diseases (e.g. *Schistosoma,* STHs,

malaria and tuberculosis), with studies generally reporting reasonably high levels of sensitivity and specificity (>90%) for visual detection of pathogens in the digitized samples. Although digital image-analysis algorithms have been extensively studied in e.g. digital pathology with samples digitized with high-end slide scanners (Bera et al., 2019, Esteva et al., 2017), they have not been widely applied for POC diagnostics. The potential to automatize the sample analysis process also in areas lacking trained experts on-site has been recognized, and a few studies have applied image-analysis methods to samples digitized in POC field settings. To date however, the work on this field is limited, with only a few studies evaluating these techniques for a limited number of applications - mainly identification of infectious pathogens, such as tropical parasites and tuberculosis (Vasiman, A., Stothard, J.R. & Bogoch, I.I. 2019).

A major field where improved diagnostics is urgently needed and early diagnosis is crucial is oncology (Bender, 2014). During the coming decade, the cancer incidence is predicted to double in sub-Saharan Africa. In most low-resource settings, patients diagnosed with cancer typically have advanced diseases (Morhason-Bello et al., 2013). Confirmed histological diagnosis is the initial step and cornerstone in cancer treatment, but in most areas here, laboratory services are severely limited and the number of practising pathologists very low - even under one per 1,000,000 people (Adesina et al., 2013). Breast cancer is the most common cancer in women (Bray et al., 2018), and a leading cause of cancer-related mortality in low-resource countries where access to diagnosis is limited. In Study I, we described a diagnostic pipeline for automatized breast cancer histopathology, and applied it for the assessment of breast cancer hormonal receptor (ER) status in IHC-stained tissue samples, scanned with a small-sized POC digital microscope. Our findings suggest that automatic digital assessment of ER-expression in breast cancer samples, scanned with this type of device yields results that are comparable to both visual analysis of samples and to image analysis of samples, scanned with high-end laboratory equipment. Here, the incorrect classification of negative samples as weakly positive represented the principal challenge for the system; a task which has previously been recognized as a source of variability also with conventional methods (Hammond et al., 2010). Notably, we observed no false-negative samples with the system described, suggesting feasibility as a diagnostic support tool in the determination of tumour characteristics, which for this application is a vital step when identifying patients likely to benefit from hormonal therapies.

The results from Study I are in line with the findings from Study II, where we described how the remote assessment of samples (FS) from breast cancer surgeries yielded similar results regardless of whether a laboratory-grade slide scanner or the low-cost microscope prototype was used for digitization of slides. The assessment of sentinel lymph node status during breast cancer surgeries is a routine procedure, as the presence of axillary metastases is a strong prognostic factor and a sign of advanced disease, which might therefore indicate a need for more extensive treatment interventions (Cardoso et al., 2019, Ditsch et al., 2019). FS represents the most common method of detecting axillary lymph node metastases during the surgery, but requires access to pathologist services on-site. Certain locations utilize digital microscopy with whole-slide scanners to overcome this by enabling sample analysis by remotely located experts (Thorstenson, Molin & Lundstrom, 2014), and in Study II, we describe how the same is possible with a device which can be manufactured orders of magnitude cheaper. In the Study, we achieved levels of sensitivity and specificity which are comparable to analysis of samples from with a traditional high-end slide scanner. The principal challenge here was the detection of small metastases in samples where only the morphological staining was available (toluidine blue), which is known to be challenging also with conventional microscopy (Chao, 2004), but overall few major discrepancies were observed. Notably, digital algorithms based on deep learning have been applied for the detection of metastases in conventional high-end WSIs (Ehteshami Bejnordi et al., 2017), and based on the results from Study II, similar methods are likely to be applicable also to samples digitized with lower-cost equipment.

Cervical cancer represents another major global health burden. Although being highly preventable, the disease remains one of the most common and deadliest cancers in many countries (Bray et al., 2018). Pap smear-screening has drastically reduced the incidence and mortality of cervical cancer in high-resource areas (Saslow et al., 2002), but as screening coverage is still severely limited in many countries, cervical cancer remains one of the most common cancers in women here. As the analysis of Pap smears is a time-consuming and labour-intense process with relatively low sensitivity and reproducibility (Sørbye et al., 2017), the development of automatized systems has been studies for decades (Bengtsson, Malm, 2014). The development of completely automatized systems has been challenging due to the complexity of samples, and the current systems allow mainly pre-screening of slides with high-end equipment and/or LBC-processors. Deep learning-based algorithms have been applied also for detection of cervical-cell atypia in images from digital

pap smears with promising results (L. Zhang et al., 2017), but mainly using small-sized, cropped images from digital samples, meaning that the results are not directly applicable clinically in situations where the whole slide needs to be analysed (William et al., 2018). In Study III, we describe a digital diagnostic system where Pap smear whole slides are digitized with a small-sized slide scanner, uploaded to a cloud server and analysed with a deep-learning model, trained to detect atypical, potentially precancerous lesions. We implement this technology at a peripheral clinic in Kenya in a region with high cervical cancer prevalence and low screening coverage for the analysis of routine Pap smears. Our findings suggest that detection of atypical slides with the technology is feasible with high sensitivity and specificity for lesions that are likely to warrant treatment interventions (e.g. low-grade or higher squamous-cellular atypia). As the overall NPV was high (~99 %), this technology shows potential especially for screening purposes. Notably, however, in this study we did not evaluate the system for detection of atypical cells of undetermined significance (ASC-US and ASC-H) or glandular atypia. As these types of cells have distinct morphological features which are likely to be resolvable by visual evaluation of the digital samples in this study, it seems reasonable to assume that this technology is applicable also for the detection of these types of atypia, although further investigation is warranted to confirm this.

Diagnostics of infectious diseases is a major application where digital microscopy has a significant potential to improve POC diagnostics, as current standards of diagnostics rely heavily on light microscopy (Knopp et al., 2012). Rapid and accurate diagnosis is essential in treatment and clinical management of parasitic diseases, but the lack of access to trained personnel and laboratory infrastructure compromises access to adequate diagnosis in the most affected areas. The need for improved testing for diseases such as neglected tropical diseases and malaria is enormous; annually hundreds of millions of blood smears (for malaria), stool samples (for helminth infections) and urine samples (e.g. schistosomiasis), are examined for parasites, but the need for more is significant (O'Meara et al., 2010). Accurate parasite count is essential not only for diagnosis and disease staging, such as when assessing level of parasitaemia in e.g. malaria, but also for disease management and control (such as in the monitoring of MDAs). During the last decades, studies have demonstrated how inexpensive, handheld digital microscopes can be utilized to diagnose various parasitic diseases, such as schistosomiasis, STHs, malaria and tuberculosis in field settings (Vasiman, A., Stothard, J.R. & Bogoch, I.I. 2019), suggesting a

significant potential for this technology to provide novel tools for diagnostics of these conditions. Out results from Study IV complement the findings from earlier studies, and demonstrate how a deep-learning algorithm can be applied to accurately detect the most common tropical parasites in digital samples, scanned with a low-cost POC device. We achieved generally high levels of sensitivity (83 - 100 %) with the system described, which is similar to what has been achieved with manual assessment of digital samples for the same diseases (Saeed, Jabbar, 2017). Furthermore, we demonstrate how results can be innovatively presented in a single panel to allow rapid manual verification of results (Figure 19), compared to visually reviewing thousands of microscopic FOVs. NTDs remain a significant global health burden, with a recognized need for improved POC diagnostic tools (PATH, 2015) and our results support the conclusions from earlier work in suggesting that this technology is usable for this purpose.

Overall, the field of computer vision and AI for medical image-based diagnostics has evolved rapidly during recent years, mainly with the rise of medical AI's with deep neural networks, which enable highly-efficient analysis of medical images for a variety of applications, ranging from automatized image segmentation and classification to detection and quantification of features of interest. In this work, we have evaluated this type of digital image-analysis only for a limited number of applications, but as discussed in previous sections, by disseminating platforms to enable general-purpose digital microscopy at the POC, these methods are likely to be applicable also for various other diagnostic applications. Notably, apart from facilitation the traditional sample analysis process, recent research findings demonstrate how deep learning-based analysis of samples can extract information from digital samples in completely novel ways – e.g. by allowing predictive decision-making based on tissue morphology, 3D tissue modelling and news ways of interpreting high volumes of multimodal data (Bera et al., 2019). Assuming that the image quality achievable with POC imaging devices, such as the ones studied here, is comparable to laboratory-grade equipment for certain applications, it is reasonable to assume that these methods could be applicable also for POC diagnostics outside high-end laboratories.

Although the findings here suggest multiple opportunities to improve access to diagnostics on a global level, certain challenges exist that need to be addressed before wider-scale clinical implementation is possible. One important aspect is the practical challenges related to preparation, handling and staining of samples; all of which are vital steps for efficient POC diagnostics (Vasiman, A., Stothard, J.R. & Bogoch, I.I. 2019).

Interestingly, studies have demonstrated how deep-learning models can perform "virtual" tissue stainings on native samples (Rivenson et al., 2019), thus potentially reducing the need for e.g. extensive staining protocols. To allow clinical usage, certain technical issues are crucial to address. Examples of these for current systems include ensuring support for imaging of sufficiently large sample areas (multiple FOVs), ease-of-use with minimal training and sturdy construction to withstand rugged field conditions. In clinical situations, the number of samples needed to be analysed can be large, which puts increased demand on the slide processing workflow in terms of digitization (turnaround time for slide scanning), storage and uploading (local storage capabilities, network performance and price) and computational resources for digital image analysis software. Importantly, in terms of per-sample costs, even though the device manufacturing costs can be significantly reduced by choosing more cost-efficient components, more expensive alternatives with increased longevity could provide more viable alternatives over a longer time period, e.g. in settings where very large numbers (e.g. hundreds of thousands) of slides need to be digitized over longer periods of time. One issue with both novel hardware solutions (e.g. the current generation of POC digital microscopy devices) and software methods (e.g. medical AI's) is the lack of standardizations, external validation data and regulatory approval methods. Notably, efforts are being made to overcome this, with the UN currently working on developing international standardized evaluation processes for health AI models (Wiegand et al., 2019). Currently, as a majority of studies on these technologies are relatively limited and have been conducted in controlled conditions, there is a critical need for translating the technologies from research to clinical applications. For this, larger studies that reach beyond demonstrating feasibility to expanded population studies with validated clinical trials are required. Following this, coordinated efforts and industry support are crucial to actually implement the technologies into clinical practise, by e.g. developing strategies for manufacturing and business commercialisation to finally reach the intended populations. This so-called 'last mile problem' is a well-recognized, extremely challenging problem for a number of emerging technologies for global health applications (Chao et al., 2014).

In conclusion, this work demonstrates how modern technological advancements can be utilized to facilitate microscopy diagnostics at the POC. The methods studied here could provide novel digital diagnostic tools, which are not only feasible to use for diagnostics of various common diseases (e.g. applications in cancer histopathology, infectious diseases and analysis of cytological samples), but also implementable outside high-end digital laboratories, where the actual need for improved diagnostics is highest. Modern image-analysis algorithms are well-suited to facilitate the analysis of digital samples, and can improve the sample analysis process in a number of ways, which could be of particular value when access to trained medical experts is limited. These findings are encouraging steps in the effort to develop novel solutions for fast, accurate and reliable digital diagnostics at the POC. As this field is rapidly developing, further technological advancements are likely to translate into improved diagnostic performance, but this does not seem to be the breakthrough needed for success. Instead, to bridge the gap between academia and clinical work, coordinated efforts between different stakeholders are required to properly develop, scale production and implement these technologies. In this way, these already existing technologies could more efficiently be translated from research laboratories to improving access to diagnostics on a global level.

7 CONCLUSIONS

In this doctoral thesis, we have described, developed, implemented and evaluated novel diagnostic solutions to digitize microscopy samples at the point of care for analysis either visually or with automatized digital algorithms. Based on the findings in the original research articles, the main conclusions are:

1. The image quality achievable with a low-cost, point-of-care digital microscopy platform is sufficient for manual or digital assessment of breast cancer hormone receptor (ER) status.
2. A low-cost, point-of-care digital microscope can provide a platform to allow remote assessment of intraoperative lymph node frozen sections for detection of breast cancer metastases.
3. A system based on point-of-care digital microscopy with deep-learning based artificial intelligence can be implementable in peripheral settings and used to detect cervical-cellular atypia in digitized Pap smears.
4. Detection and classification of the globally most common neglected tropical diseases is feasible by utilizing low-cost, digital microscopy and deep learning-based artificial intelligence.

TIIVISTELMÄ (Summary in Finnish)

Riittämätön diagnostiikka ja diagnostisten testien heikko saatavuus on globaalinen ongelma, joka johtaa monen yleisen sairauden alidiagnosointiin. Monilla alueilla laboratoriopalveluiden ja lääketieteellisten asiantuntijoiden saatavuus on hyvin rajallista, kuten esimerkiksi Saharan etelänpuolisessa Afrikassa, jossa patologeja on usein vähemmän kuin yksi per miljoona asukasta. Viime vuosien teknologinen kehitys on mahdollistanut edullisten, pienikokoisten digitaalisten mikroskooppien rakentamisen, jotka soveltuvat käytettäviksi myös kenttäolosuhteissa (’Point of care’). Koska mikroskooppinäytteiden digitalisoiminen on perinteisesti vaatinut isokokoisia ja kalliita laitteita, menetelmä on pääasiassa ollut rajoittunut isoihin laboratorioihin. Digitaalisen mikroskopoinnin tuominen laboratorioiden ulkopuolelle mahdollistaa näytteiden analysoinnin digitaalisilla (esimerkiksi ns. ”keinoälyyn” perustuvilla) menetelmillä myös kenttäolosuhteissa, jossa nopea ja luotettava diagnostiikka on useimmiten erityisen tärkeä.

Väitöskirjan tavoitteena on tutkia miten potilasläheistä digitaalista mikroskopointia, yhdistettynä keinoälyyn ja konenäköön perustuvaan digitaaliseen analysointiin, voidaan soveltaa tehostamaan diagnostiikkaa erityisesti potentiaalisissa käyttötarkoituksissa alueilla, joissa lääkäri- ja laboratoriopalveluita ei ole riittävästi saatavilla.

Työssä tutkitaan erilaisia menetelmiä digitalisoida ja analysoida mikroskooppinäytteitä potilasläheisesti ja analysoida niitä sekä visuaalisesti, että käyttäen digitaalisia menetelmiä. Työ on jaettu neljään alaosaan, joissa tutkitaan erilaisia mahdollisia teknologian käyttöaiheita. Nämä ovat: 1) syöpäkudospatologia (kudoksen hormonireseptoripositiivisuuden määrittäminen), leikkauksen aikana tapahtuva patologia (jääleikkeiden analysointi etäpesäkkeiden löytämiseksi), sytologisten näytteiden analysointi (Papa-näytteiden analysointi) sekä yleisimpien troppisten parasiittitautien diagnostiikka (’Neglected tropical diseases’).

Tuloksemme osoittavat, kuinka erilaisia mikroskooppinäytteitä (syöpäkudosnäytteitä, sytologisia näytteitä sekä parasitologisia näytteitä) voidaan digitalisoida diagnostisiin tarkoituksiin käyttäen pienikokoisia, edullisia laitteita, jotka soveltuvat käytettäviksi kenttäolosuhteissa. Lisäksi tuloksemme osoittavat kuinka käyttäen digitaalisia algoritmeja voidaan tehostaa näytteiden analysointia ja mm. määrittää

kudosvärjäyksiä, tunnistaa syövän esiasteita ja identifioida patogeenejä näytteistä tarkkuudella, joka vastaa perinteisiä diagnostisia menetelmiä.

Yhteenvetona tuloksemme osoittavat miten teknologista kehitystä voidaan hyödyntää mahdollistamaan mikroskooppinäytteiden digitalisoimisen potilasläheisesti myös kenttäolosuhteissa. Tämä mahdollistaa digitaalisten algoritmien ja keinoälyn käyttämisen tehostamaan ja automatisoimaan näytteiden analysointia. Löydöksillä on merkitystä erityisesti uusien diagnostisten menetelmien kehittämisessä, jotka soveltuvat käytettäviksi myös perinteisten laboratorioiden ulkopuolella. Lisäksi täällä esitetyt menetelmät soveltuvat todennäköisesti käytettäviksi myös muiden sairauksien diagnostiikkaan, joka tällä hetkellä perustuu valomikroskopointiin.

SAMMANFATTNING (Summary in Swedish)

Bristande åtkomst till medicinsk diagnostik är ett signifikant globalt problem som resulterar i att många vanliga, behandlingsbara sjukdomar förblir underdiagnostiserade. I vissa områden är tillgängligheten av laboratorietjänster och medicinisk personal kraftigt begränsad, liksom exempelvis i subsahariska Afrika där det totala antalet patologer ofta är lägre än en per miljoner invånare. Under det senaste decenniet har den tekniska utvecklingen möjliggjort utvecklingen av portabla, förmånliga instrument för att digitalisera biologiska prov även i fältmiljö; något som traditionellt begränsats till högklassiga laboratorier på grund av kravet på avancerad och dyr teknik. Genom att möjliggöra patientnära (’point of care’) digitalisering av prov kan avancerade digitala metoder, som provanalys med ’artificiell intelligens’, tillämpas även i fältmiljö – dvs. där behovet av förbättrad diagnostik är störst.

Målet med avhandlingen är att undersöka hur förmånlig, patientnära digitalmikroskopi, kombinerat med digital bildanalys och artificiell intelligens, kan tillämpas för att effektivera rutinmässig mikroskopidiagnostik med tyngdpunkt på eventuella användningsområden i lågresursmiljöer.

I arbetet beskriver, implementerar och utvärderar vi olika metoder för patientnära digitalisering av mikroskopiprov och analys av proven både visuellt och med automatiserade, digitala metoder. Arbetet är uppdelat i olika områden som undersöker teknologin för olika användningsområden. Dessa är: 1) onkologisk vävnadspatologi (bestämning av hormonreceptorstatus), 2) analys av intraoperativa vävnadsprov (fryssnitt för detektion av metastaser), 3) analys av cytologiska cellprov (Papa-prov) samt 4) diagnostik av de vanligaste tropiska parasitsjukdomarna (’neglected tropical diseases’).

Resultaten visar hur digitaliseringen av mikroskopiprov är möjlig med miniatyriserade digitalmikroskop som lämpar sig för användning i fältmiljö, med en tillräcklig bildkvalitet för medicinsk diagnostik. Utöver detta kan provanalysen effektiveras med hjälp av automatiserad digital bildanalys, för att ex. mäta nivåer av vävnadsfärger samt identifiera premaligna cellförändringar samt parasiter i proven.

Sammanfattningsvis tyder resultaten på att metoderna här är lämpliga för patientnära mikroskopidiagnostik av ett flertal olika sjukdomar. Genom att tillämpa moderna digitala bildanalysmetoder kan provanalysen automatiseras och effektiveras.

Resultaten är betydande steg i utvecklingen av digitaldiagnostiska metoder som är användbara även i områden utan tillgång till högklassig laboratorieinfrastruktur. Utöver detta är metoderna beskrivna här sannolikt även möjliga att tillämpa för diagnostik av ett flertal övriga sjukdomar vars diagnostik för tillfället baserar sig på ljusmikroskopi.

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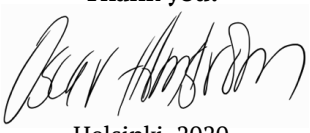

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