NPL
National Physical Laboratory

NPL REPORT AS 102

METROLOGY FOR DIGITAL PATHOLOGY

DIGITAL PATHOLOGY CROSS-THEME PROJECT REPORT

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MARCH 2021

Metrology for Digital Pathology

Digital Pathology Cross-Theme Project Report

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ISSN 1754-2928

https://doi.org/10.47120/npl.AS102

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CONTENTS

GLOSSARY AND ABBREVIATIONS

Term	Definition
AI	Artificial Intelligence
AR	Augmented Reality
BSI	British Standards Institute
CNN	Convolutional Neural Networks
CRO	Contract Research Organisation
DICOM	Digital Imaging and Communications in Medicine: A standard for capturing and exchanging medical imaging data.
DP	Digital Pathology
FDA	U.S. Food and Drug Administration
GPU	Graphics Processing Unit
H&E	Haematoxylin and Eosin stain: The most frequent stain type used in histopathology
IA	Image Analysis
ISCF	Industrial Strategy Challenge Fund
ISO	International Organisation for Standardisation
IHC	Immunohistochemistry
ISH	In-situ hybridisation
LIMS	Laboratory Information Management System
MDT	Multidisciplinary teams: A group review of a case by clinicians from different specialties
MSI	Mass Spectrometry Imaging
necroscopy	Examination of dead body
NHS	National Health System: The main healthcare provider in the UK
NPIC	Northern Pathology Imaging Co-operative
NPL	National Physical Laboratory
OME-TIFF	An image file format
omics	Collective term for biology disciplines that study various molecules including genomics, proteomics, transcriptomics metabolomics and many others.
PathLAKE	Pathology Image Data Lake for Analytics Knowledge & Education: One of UK's five Centres of Excellence in digital pathology and medical imaging funded by ICSF
QA	Quality Assurance: All procedures and activities aimed to achieve and maintain a specified quality of product
QC	Quality Control: Procedures to monitor the testing method and test results to ensure appropriate test system performance
RCPath	Royal College of Pathologists
SOP	Standard Operating Procedure
TGCA	The Cancer Genome Atlas
VAMAS	Versailles Project on Advanced Materials and Standards
VR	Virtual Reality
WSI	Whole Slide Imaging
XML	eXtensible Markup Language: A structured text data format to store and transport data that is human- and machine-readable

EXECUTIVE SUMMARY

Pathology underpins over 90% of healthcare decisions and is undergoing digital modernisation, increasingly relying on novel imaging and data analysis methods. The pressure on the UK pathology services has been amplified by the COVID-19 pandemic. While digitalisation of pathology services can help alleviate this pressure, many digitalisation solutions are premature, having been based on unstandardized processes, uncalibrated instruments, black-box AI tools and low-quality data. Metrology guidance on safe practices in use of Artificial Intelligence tools, industry-wide data and metadata standards, techniques to address variabilities in the image acquisition, and the training of pathologists are key to improving patient outcomes through faster and more precise, more robust diagnostics.

To support the Industrial Strategy Challenge Fund investment in the Digital Pathology Centres, NPL launched a Digital Pathology interdisciplinary project that commenced in 2020. This report presents the findings of the knowledge gathering exercise that concluded the first phase of the project. The findings include a) a literature review (section 2.1), b) results of an online survey to identify metrology needs in Digital Pathology (sections 2.2 and 6) and c) the outcomes of an NPL workshop with Digital Pathology experts that took place in January 2021 and attracted over 40 attendees from the NHS, pharma, equipment and software vendors, as well as academia (sections 2.3 and 3).

Significant barriers to adoption of digital technologies in pathology and AI-based analytics were identified during the workshop and are presented in section 3. The workshop has brought to light urgent needs for data and metadata standards, interoperability, quality control measures and accreditation, equipment calibration, development of trustworthy AI tools and training of pathologists in these areas. The experts' suggestions on how NPL can help address these needs are summarised in section 4.

Future work described in section 5 will focus on addressing the prioritised challenges in the area by working with key partners including NHS Digital, The Royal College of Pathologists, The British Standards Institute and the Digital Pathology Centres of Excellence. Engaging with these stakeholders throughout the project aligns with NPL's strategic priorities and foresighting in the intelligent use of data, early detection of priority diseases, and confidence in decision making, whilst supporting the UK government's £50m investment to support Digital Pathology through the ISCF.

1 INTRODUCTION

Pathology is the backbone of diagnostic medicine, contributing to 95% of clinical pathways. It plays a crucial part in cancer prevention and treatment through screening, diagnosis, intra-operative assessments, and post-treatment monitoring. A Cancer Research UK study published in 2016 forecasts a 42% growth of cancer incidence between 2015 and 2035 (Smittenaar et al. 2016). This data is consistent with the findings of the Royal College of Pathologists that report a 4.5% year-on-year increase in demand for pathology services since 2007 (The Royal College of Pathologists n.d.). The growing diagnostic demand is accompanied by a 17% yearly increase in the number of delayed cancer cases, i.e. cases that breach the 6-week time-to-treatment target. The majority of these delays are caused by a lack of access to histopathology services (Williams, Bottoms, and Treanor 2017) leading to worse patient outcomes.

The COVID-19 pandemic has amplified already mounting pressures on pathologists. Studies estimate that between 5% and 15% of avoidable cancer deaths are caused by delayed diagnosis (Maringe et al. 2020). Digitalisation of pathology will help alleviating this pressure, improve the UK's healthcare system, its pandemic resilience, and allow faster access to expert diagnosis (Figure 1). Digital whole slide imaging (WSI) is a key enabler of large-scale image analysis that allows establishment of institutional, national and international virtual biobanks, accelerating discovery and diagnosis. Recent studies have demonstrated that digitalisation of pathology services increased throughput by 21% of cases per year (Retamero, Aneiros-Fernandez, and Del Moral 2020).

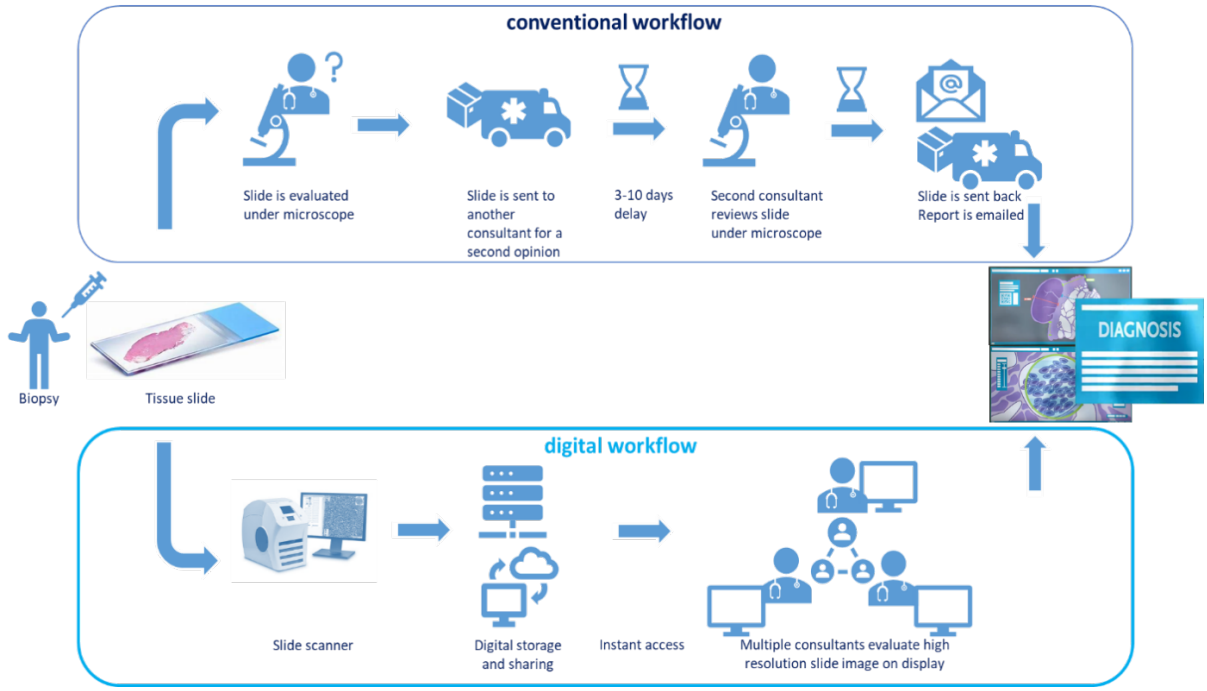

Figure 1: Conventional (top) vs digital pathology workflow (bottom).

However, the backlog of patients caused by the pandemic may force many services into premature adoption of AI, where decisions systems are based on unstandardized processes, "black box" software, lack of data provenance or traceability, and unknown uncertainties.

The NPL Digital Pathology interdisciplinary project set out to identify how metrology and metrological data science can help to overcome the key barriers to adoption of Digital Pathology: data interoperability, reproducibility of workflows, confidence in existing and novel analysis methods as well as applications of novel imaging techniques and “omics”. The NPL Digital Pathology survey conducted in January 2021 revealed that users struggle to access the data required for diagnosis, as it is scattered across multiple platforms and systems. As the UK’s National Measurement Institute NPL has an opportunity to improve confidence in data acquisition and analysis tools, improve existing workflow workflows, reduce time-to-diagnosis, and ultimately support improved patient outcomes.

2 GAP ANALYSIS

2.1 LITERATURE REVIEW

Digitalisation of pathology has been ongoing for nearly two decades, and in that time a deeper understanding of the barriers and challenges has been obtained (Pantanowitz et al. 2018). Here, an outline is given of the knowledge and technological gaps identified in the literature.

2.1.1 GENERAL

The uptake of Digital Pathology is gathering speed worldwide and in the UK. As of 2017, 60% of surveyed UK pathology centres had access to WSI scanners or WSI workstations (Williams et al. 2018). Various guidelines to support the rollout have been produced, and challenges accompanying or hindering Digital Pathology uptake have been highlighted. Since 2016 the NHS Digital Diagnostics Data Service has been “improving data quality and interoperability standards in pathology, imaging, endoscopy, physiological measures and genomics health services” (NHS Digital 2020). The Data Service supports the reporting of pathology test results from NHS labs to GPs. The “Digital First” report was published by NHS England in 2014 and highlighted the use of digital systems and process in pathology across the country. Also in 2016, the FDA announced guidance for data that should be submitted for regulatory evaluation of a Digital Pathology WSI system (Food and Drug Administration 2016). The document included guidance on the WSI system components throughout the pipeline and includes tests that should be carried out to validate any software outputs.

UK’s efforts towards quality assurance in pathology included a guidance document provided by the Royal College of Pathologists that points to the relevant information in the ISO 15189:2012 standard, which outlines an approach to the assessment of uncertainty of measurement for cellular pathology laboratories (Helliwell T, Giles T, Fensome-Rimmer S. 2015).

General challenges to the uptake of Digital Pathology are varied. A 2017 review of clinical Digital Pathology found that acceptance by pathologists was a barrier to Digital Pathology uptake (Griffin and Treanor 2017), while trainee histopathologists were concerned about maintaining competence on both traditional microscopy and Digital Pathology (Browning et al. 2020).

In terms of finances and equipment, long term data storage is a challenge, as WSI data can require many terabytes of storage (Colling et al. 2019). Interestingly, a survey of the UK pathology community in 2018 found that financial rather than safety costs were a bigger barrier to uptake than safety concerns (Williams, Bottoms, and Treanor 2017). During the

COVID-19 pandemic, there was a sharp uptake in Digital Pathology, and half of surveyed respondents reported that Digital Pathology facilitated maintenance of diagnostic practice (Browning et al. 2020). Many of the challenges faced were around home ‘set-up’ (i.e. internet speed, suitable screen), and an earlier study mentioned similar challenges with NHS internet/computers.

A Best Practice Guide by the Royal College of Pathologists in 2018 provides a comprehensive overview of Digital Pathology challenges (Cross et al. 2018) such as display quality issues, display maintenance, legal issues when sending images outside the UK for diagnosis, differences in appearance between scanned and physical slide.

2.1.2 Sample Processing

Sample processing in pathology comprises multiple stages depending on the test, tissue, and stain type. For haematoxylin and eosin (H&E) stain that prevails in human tissue examinations (Chan 2014), it includes specimen grossing, tissue fixation, embedding of tissue blocks in paraffin, microtomy, tissue fixation, staining and slide preparation. Every step of this process is subject to variations that are propagated through the imaging process to diagnosis. Slide quality assurance is performed manually and is a subject to the experience of the assessor. Studies have shown that variations in sample processing are a major contributor to image quality fluctuations and may render the images unusable for conventional diagnosis or computerised assessment. Staining variability also called “batch variability” has been highlighted as a major obstacle in producing consistent and comparable images (Aeffner et al. 2019), and attempts to address this issue using alternative imaging technologies and image post-processing have been undertaken (Majeed et al. 2019).

2.1.3 Equipment Calibration

A whole slide imaging scanner is a high-throughput microscope that scans the stained tissue slides and produces high resolution images between 1-10 Terabyte in size. WSI scanners typically include mechanical stage to feed the slides, light source, optics, and a digital camera sensor. In 2015, the USA Food and Drug Administration authority issued a set of recommendations on technical quality assessment of WSI devices and its components (Food and Drug Administration 2016) to help evaluate WSI scanners and maintain consistent image quality during their lifetime. However, these recommendations are non-binding and would be difficult to follow in a clinical setting with no specialised equipment and lack of resource.

To date, the scope and frequency of WSI calibration and routine tests vary between vendors and laboratories. In the UK, RCPath report of 2018 points out that, while WSI are medical devices that should undergo ongoing quality assurance, and subjective perception of image quality cannot indicate the image is “fit-for-diagnosis”, the research in the calibration area “is sparse”. The relevant calibration areas highlighted in the report include (1) dimensionality, (2) illumination and (3) colour (Cross et al. 2018). Other WSI scanner features that are thought to have significant impact on image quality and diagnostic assessment include the amount of ambient light, depth of field, inability to polarise the slides etc. (Browning et al. 2020).

The most widely recognised challenge in Digital Pathology is colour calibration that includes (1) internal colour calibration which involves standardisation and correction of the scanning process itself and (2) external colour calibration that focusses on the standardisation of the display, accounting for the monitor’s effect on perceived colour and the viewing environment.

A review of colour in Digital Pathology noted that colour management in Digital Pathology is challenging due to the lack of standards (Clarke E and Treanor D, 2017). The same review also highlighted that colour variation is substantial in pathology, and that digitisation of the slide introduces further lack of colour control and compounds the issue.

2.1.4 Image Analysis, Artificial Intelligence & Machine Learning

General barriers to AI adoption in pathology include lack of transparency and interpretability, computational expense (in terms of GPUs costs), networking issues posed by large data volumes and adversarial attacks (Tschuchnig, Oostingh, and Gadermayr 2020).

Since the implementation of AI tools in histopathology is entirely reliant on the digitalisation of pathology laboratories, it is vital that the gaps in other Digital Pathology areas such as sample processing, equipment calibration and QA as well as data integration are addressed prior to AI/ML system deployment (Colling et al. 2019). A review of machine learning methods for histopathological image analysis highlighted the following problems: very large image size, insufficient labelled images for training data, lack of consensus on optimal equipment settings for diagnosis (i.e., what magnification should be used), colour variation and artefacts (Komura and Ishikawa 2018). Challenges of AI & ML implementation also include factors such as financing scanners and software, and long-term data storage. It has been noted that the field is missing the infrastructure to scan, catalogue, and store large collections of WSI data that would be required for AI applications (Pantanowitz et al. 2018).

Further challenges hindering AI implementation in Digital Pathology is the granularity and the quality of data annotation, its format, and labelling concordance between experts. The agreement between pathologists varies with cancer type, with some cases having less than 50% concordance, and can be affected by properties such as tissue density (Elmore et al. 2015). A review noted that lack of detailed annotation or labelled data is a hindrance to uptake of deep learning methods in whole slide imaging (Dimitriou, Arandjelović, and Caie 2019). The same review also noted that the lack of a universal image format hinders the curation of large datasets suitable for deep learning. Slide and image artefacts as well as colour variation were also highlighted as a barrier to successful deep learning with WSI data. The lack of regulation-driven evidence-based validation is a significant barrier to the rollout of Digital Pathology systems, and the need for data integration of commercial and open-source data formats has been noted (Colling et al. 2019).

Interpretability and trust in AI-based analysis have been highlighted as significant challenges, including the need for interpretability methods for Digital Pathology deep learning and rapid classification for pathological diagnosis during surgery (Komura and Ishikawa 2018). Leeds Guide to Digital Pathology notes that trust in analytics is essential for deployment of Digital Pathology and note that “if it’s not measured, it can’t be improved” (Treanor and Williams 2018). Furthermore, regular implementation of AI tools by pathologists will affect daily practise, and there is a need to provide support and assessment to protect diagnostic skills (Colling et al. 2019).

Finally, IA, AI & ML methods must be able to handle artefacts safely: methods for detecting artefacts such as tissue folds are being developed (Kothari S et al., 2013) as are deep learning approaches for detecting out-of-focus regions in WSI scans (Seneras C et al., 2018).

2.1.5 Multi-Omics & Novel Imaging

Pathological investigations can either target structural information by using conventional stains such as H&E or Periodic Acid–Schiff, or other information such as genetic, proteomic or metabolomic information. Some emerging techniques for Digital Pathology aim to combine these pieces of information together providing metabolic, proteomic or genomic images of tissues to better understand complex diseases as illustrated in **Error! Reference source not found.** (Bobroff et al. 2017; Bueno et al. 2016; Cicchi 2014; Aichler and Walch 2015)

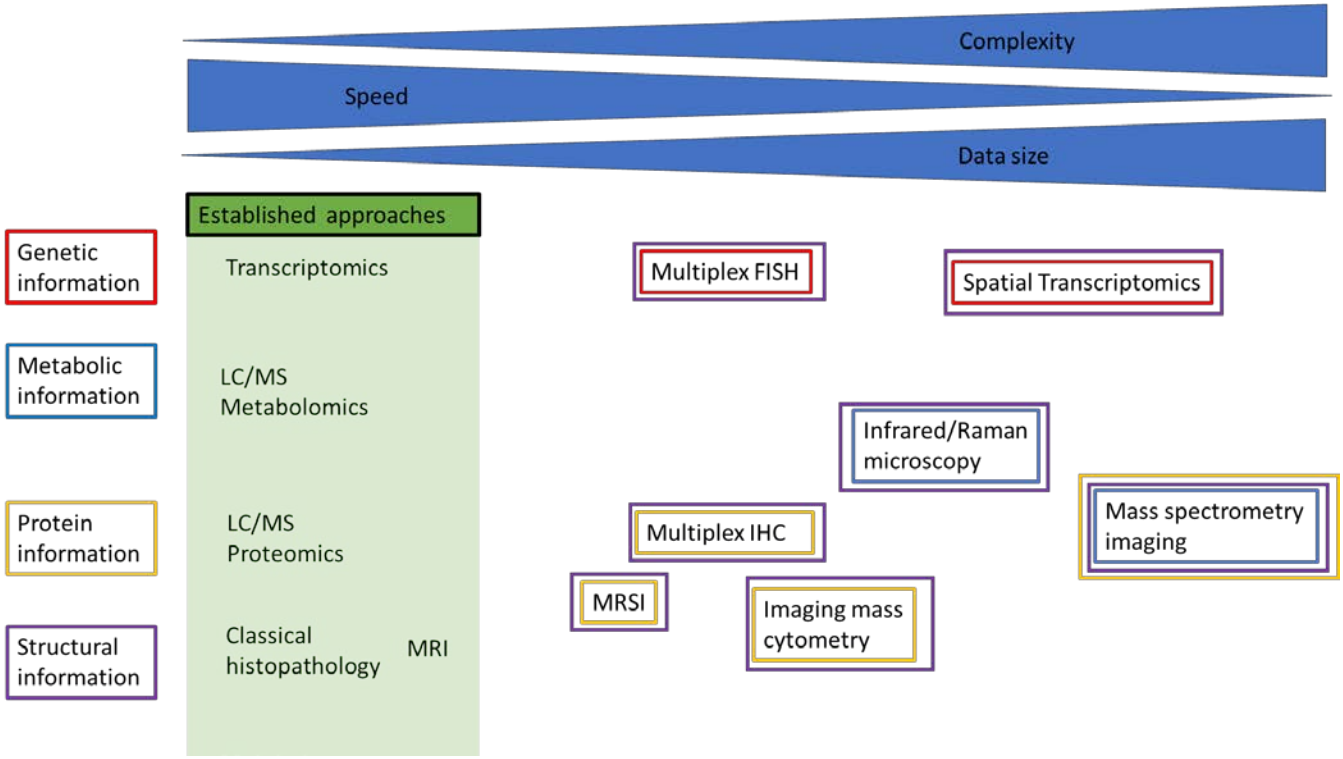

Figure 2: Insights into tissue structures provided by various multi-omics techniques.

Since multiple measurements can be made at every pixel of an image, a greater amount of information is used to diagnose a given disease offering advantages over histopathology; Firstly by using combinations of measurements it is possible to differentiate diseases where single measurands do not (Zhou et al. 2019). This can also improve accuracy of diagnosis, including tumour grading (Morais et al. 2019) and measuring intra tumour heterogeneity (Abdelmoula et al. 2016).

These techniques can also be used as precursors for in vivo diagnostic methods. The two primary examples of this are the use of Raman microscopy as a precursor for *in vitro* Raman spectroscopic diagnostics (Bergholt et al. 2011), and mass spectrometry imaging to identify molecular biomarkers that can be used in classification of disease during surgery (Balog et al. 2013). This has the potential to revolutionise disease diagnosis by providing real time

Disease classification using these methods is typically based on more directly interpretable information. For example, Sans *et al.* showed fatty acids as markers for tumour aggressiveness using mass spectrometry imaging (Sans et al. 2017). This information can then be used to develop alternative non-invasive diagnostic methods or more personalised medicine.

The major barriers to large scale clinical uptake of these analyses can be broken down into three categories: The data collection and sample preparation needed can be more complex (Goodwin 2012; Everall 2010), the data themselves are much larger and more complex meaning data analyses are more challenging (Shakya et al. 2020), and these methods are not yet fully understood within a clinical setting, and are not yet approved by regulating bodies for clinical use (Finlayson, Rinaldi, and Baker 2019).

2.1.5.1 Sample preparation, data collection and instrumentation

Instrumentation and sample preparation for multiplex and hyperspectral imaging is often more complex and expensive than histological staining and analysis. Furthermore, many of these techniques are optimised for fresh tissue analysis, whereas standard histopathology workflows use formalin fixed tissues. While there have been developments in sample processing for methods such as Raman microscopy (Hobro and Smith 2017) and mass spectrometry imaging (Carter, McLeod, and Bunch 2011), this remains a barrier to regular uptake of these approaches in a clinical setting. This is of particular relevance when considering large volumes of archived tissues which are almost exclusively formalin fixed and paraffin embedded. In addition to this, the instrumentation used in these experiments is more complex than a microscope, meaning there are more variables between datasets that need to be calibrated (Workman 2018).

2.1.5.2 Data size and complexity

Many of the techniques shown collect multiple measurements per pixel in an image. As a result, these data are typically much larger and more complex than histopathology data (Wrobel and Bhargava 2018; Krafft and Popp 2019; Palmer, Trede, and Alexandrov 2016). This requires improvements in infrastructure, and data processing methods to be able to cope with these large data before their use to be more widespread. There are many memory efficient algorithms for data analysis that have been developed for these large hyperspectral datasets, but this remains an area for continued research as the size of these datasets continues to grow (Race et al. 2013; Dexter et al. 2017).

2.1.5.3 Clinical use

There have been some examples of translation of these techniques into clinical use, but these remain rare. As their use become common within a clinical environment, there will be a growing need to have approved and standardised methods for each technique as well as knowledge on which methods to apply to a given problem. Developments in knowledge transfer and understanding of these methods will enable clinicians to make full use of the suite of techniques that are available to make the most accurate and comprehensive diagnosis available.

2.1.5.4 Summary

New technologies for Digital Pathology are being developed and refined every day, and the pose exciting and potentially revolutionary improvements on the standard approaches used. Despite this there are many barriers that remain to be addressed before widespread clinical application of new technologies for Digital Pathology.

2.1.6 DATA INTEGRATION

Solid data integration underpins Digital Pathology and requires standardised data and metadata formats alongside minimum metadata requirements. A general review of clinical Digital Pathology by Griffin and Treanor (2017) noted that it is uncertain, how data from various sources such as H&E, immunohistochemistry or radiology can be integrated and visualised, and that it presents a unique bioinformatics challenge. A multi-centre Digital Pathology study conducted in Spain found that a key hindrance to its uptake was a lack of sample tracking, or a system that does not permit bidirectional transfer of information (Retamero J A et al., 2020).

Digital Pathology data sharing would benefit the field, and it has been noted that the lack of standardisation or widespread adoption of a standard such as DICOM will delay the integration of any vendor neutral archive into WSI applications such as systems for primary diagnosis or surgical pathology (Pantanowitz L et al., 2018). Others have highlighted the need for standardised open source data formats (Colling et al., 2019).

The DICOM working group 26 is developing a medical imaging standard for WSI, but notes that it is difficult for its members to advance the work without an allocated funding stream alongside their fulltime jobs (Clunie et al. 2018). Several of the Digital Pathology reviews mentioned in this section have commented that widespread uptake of a standard in Digital Pathology such as DICOM would be very beneficial. The “DICOM in Digital Pathology” white paper (Sectra Medical 2017) states that the lack of a standardised file format in DP decreases workflow efficiency and inhibits communication between slide scanners and surrounding IT systems regardless of vendor. A study that carried out a multi-site multi-vendor implementation of the DICOM standard outlined the following challenges with respect to the successful uptake and implementation of a DICOM standard in Digital Pathology: (1) large image size complicates storage and network transmission, (2) vendors do not currently store files in DICOM format, (3) choosing optimal image compression, (4) integration of imaging and information systems and finally (5) operational challenges including IT infrastructure and storage requirements (Herman M D et al., 2018). Some of these challenges were addressed in an online WSI DICOM-PACS and viewer implementation (Marques Godinho et al. 2017) and could enable online image review, easing the pressures on pathology services caused by Covid-19 pandemic.

2.2 STAKEHOLDER SURVEY

To enrich the findings from the literature with real-life use cases and to find indicators of metrological issues in clinical and non-clinical settings, an online survey focussed on the current metrology challenges in Digital Pathology took place between November 2020 and January 2021. The survey included 24 participants from clinical and veterinary pathology, pharmaceutical companies, WSI device manufacturers and academia.

The survey indicated that most users rely on both WSI and non-WSI sources of information such as patient records, study protocols, radiology, laboratory results and omics imaging to make a diagnostic decision. In terms of imaging, other modalities such as ICH, immunofluorescence and brightfield microscopy are frequently used to complement WSI. Most users store WSI data in vendor-proprietary formats, and nearly all participants exchange images or glass slides with other organisations. Most users are interested in omics imaging and AI applications for image analysis, whereby transparency in AI decision making has been highlighted as a key barrier to adoption.

The detailed summary of the survey results is presented in section 6.

2.3 METROLOGY FOR DIGITAL PATHOLOGY WORKSHOP

To identify the priority needs and determine what efforts to address them are underway, a stakeholder workshop was organised by NPL Digital Pathology project team on 28th of January 2021. The workshop attracted over 40 international experts and policy makers from NHS, pharmaceutical industry, microscopy, veterinary medicine, artificial intelligence, medical informatics, and government. Keynote speakers from British Standards Institute, Northern Pathology Imaging Co-operative and GlaxoSmithKline set the scene by outlining the key issues in the field and pointing out the need for metrology support in standardisation, quality assurance, reproducibility, and trustworthiness.

During the workshop, the attendees contributed their views on how metrology could help address the prioritised challenges in five identified areas: 1) sample processing, 2) equipment calibration, 3) omics and novel imaging techniques, 4) data integration and 5) image analysis, including artificial intelligence and machine learning. The introductions to these areas and the findings of the workshop are presented in the section 3.

3 WORKSHOP FINDINGS

This section presents the findings of the breakout discussion on (1) sample preparation and processing, (2) equipment calibration, (3) image processing and analysis, (4) omics and novel imaging techniques and (5) data integration. Each topic is concluded with the list of the prioritised issues raised by the workshop participants.

3.1 SAMPLE PREPARATION AND PROCESSING

Sample preparation and processing involves many different steps which can vary dramatically. For example, different methods for sample collection, differential time to processing (time between excision and fixation), cryosectioning protocols, and staining procedures can all alter the resulting images and thus downstream image processing (Schrohl et al. 2008; Wei and Simpson 2014).
As such, sample processing presents the largest source of the variability within the Digital Pathology workflow (Marée 2017). Quality Assurance and Quality Control procedures are vital to reducing this variability. The results of the survey showed 71.4% of participants believe that QA in sample processing is either important to address as soon as possible (38.1%) or useful to address (33.3%).

For the full value of a metrology framework for Digital Pathology to be realised, the physical artefacts of the samples imaged, the experimental protocols and instrumentation used to prepare, stain, image and digitise must be well integrated.

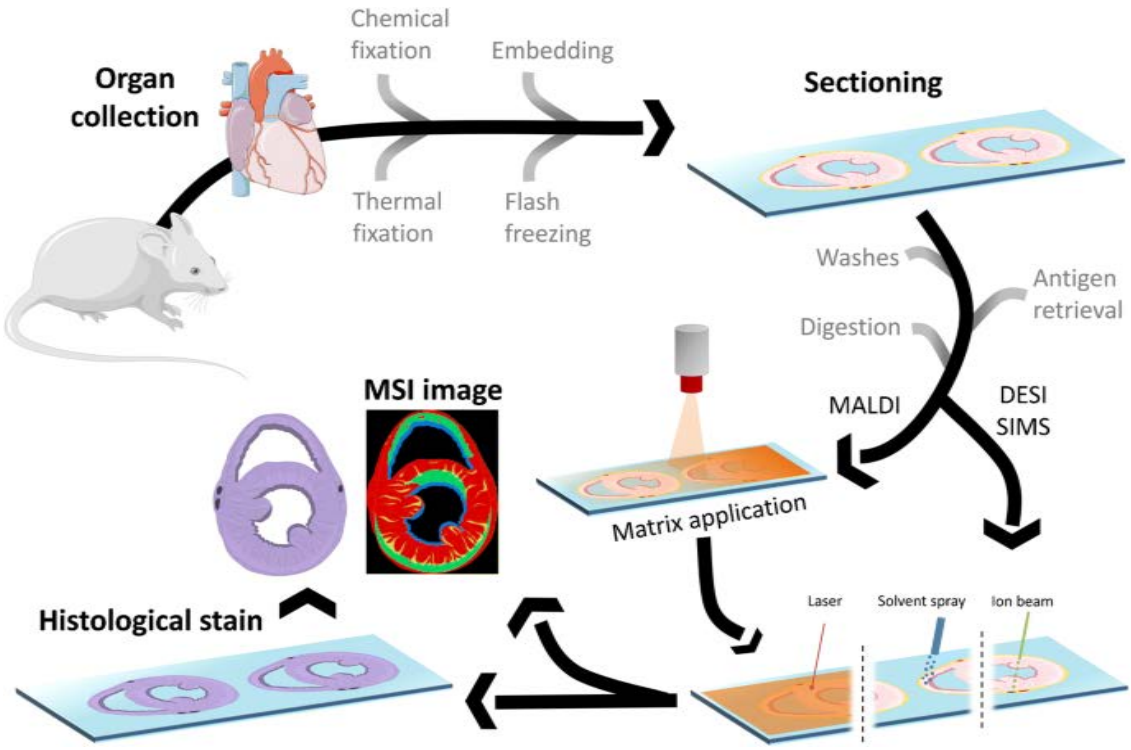

Figure 3: Sample processing for MSI & histopathology imaging.

3.1.1 IDENTIFIED GAPS IN SAMPLE PROCESSING

It has been suggested that an introduction of computerised quality assurance and quality control tools (QA QCs) would benefit the community by supporting data re-use and by monitoring the variability of system performance, data acquisition and analysis. Various views on the utility of computerised QA QCs in sample processing have been expressed and are listed below:

- Digital QA QCs are important for capturing differences in analyses undertaken using different systems (software, displays, etc.).
- Although QCs are critical, they are not well developed or taken up by the community for many modalities. While many quantitative QCs are available, they vary and are not well standardised or deployed. There is a need for QA-agnostic Standard Operation Procedures (SOPs), as the principles are often the same, but materials can vary.
- Point-of-use instrument QCs are available (Brettle et al. 2014), but they are system-specific. These system-specific QCs can be updated regularly by the manufacturer and would require uptake of new methods.
- In general, clinical instruments are better controlled than pre-clinical instrumentation. Maintaining clinical standards for high-throughput pre-clinical work is challenging.
- Historical data is not always digitised due to high time and costs required to implement the necessary procedures and frameworks to bring everything up to speed. There is a need for tools to be developed externally so uptake to a fully digital level is faster.
- Work can be outsourced to contract research organisations (CROs) at different levels, there is a desire to ensure that standardised SOPs/QA/QCs are used to make internal and external work comparable and traceable.
- Sample handling and preparation are the largest source of variability. It is therefore crucially important to develop systems to minimise this variability and to capture the information necessary for quantification of variations.

3.1.2 PRIORITISED ISSUES IN SAMPLE PROCESSING

There is a pronounced need for fit-for purpose quality metrics and standardisation routines for H&E, IHC, ISH and other tissue staining protocols. Such metrics would help in

1. Capturing and controlling variability in sample handling from necroscopy to digitisation.
2. Finding balance between discovery focussed methods and discovery/diagnosis enabling methods.
3. Addressing challenges in translating methods from in-house expertise to CRO/routine path lab partners.
4. Quantifying differences in staining needs across studies and reflect individual pathologist’s preference for staining intensity and balance.

3.2 EQUIPMENT CALIBRATION

The move to increasing digitisation in pathology has created new demands for reliable instrument calibration. Whilst pathologists may be able to accommodate variations in, for example, luminance, colour and focus when visualising samples down the microscope eyepiece, the need for sharing and comparative analysis of digital images across sites and the use of automated computational analysis places new requirements on the consistency of image data sets. Whole slide imaging systems, which can create a digital version of microscope slide, require calibration of several properties to reduce image variability. Calibration slides (for example grid patterns) can help assess the magnification, illumination uniformity and focus quality of the system and detect geometric image distortions before

acquiring images. The use of calibrated colour targets can improve the fidelity of colour representation of subsequent images of stained tissue sections. Although a number of such physical calibration standards have been developed, they often lack independent verification and validation. The detailed calibration methods employed, and the frequency with which such tests are performed, are often left to user discretion. As a result, equipment calibration is often inadequate and subjective, leading to high variance and bias. This impacts image quality and downstream diagnostic analysis.

3.2.1 IDENTIFIED GAPS IN EQUIPMENT CALIBRATION

1. Variations in the performance of microscope systems drives the need for calibration and standardisation. A lack of robust calibration methods currently limits the reliability and comparability of image-based analysis in Digital Pathology.
2. Measurements of colour fidelity, image quality / focus, geometric distortion, luminance, depth of field etc. need to be standardised in order to avoid errors in downstream diagnostic analysis (for example based on machine learning).
3. Recommendations for quality metrics and documentary standards should be provided by an independent organisation.
4. The Digital Pathology community often lacks independently verified physical calibration artefacts.
5. Absolute (traceable) calibration of instruments should be the ultimate goal in order to reduce interlaboratory variability, subjectivity and bias. However, calibration to improve repeatability of a given platform is also valuable when traceable calibration is not feasible.
6. The need to integrate data from emerging analytical techniques (imaging and non-imaging) will create increased requirements for traceable calibration of instruments used in Digital Pathology.

3.2.2 PRIORITY ISSUES IN EQUIPMENT CALIBRATION

1. There is a need for improved calibration methods across the different imaging modalities used in Digital Pathology.
2. Differences in the methods and frequency of instrument calibration across the community lead to significant variations in results. This can be addressed by establishing more widely standardised calibration methods and, where possible, ensuring traceability.
3. Impartial quantification and testing from an institution such as NPL is important to validate methods and physical calibration artefacts and promote standardisation in the field.

3.3 IMAGE PROCESING AND ANALYSIS

Computational pathology plays an increasingly important role in pathology workflows and has the potential to improve workflow efficiency and diagnostic quality. Furthermore, AI and ML applications in computational pathology can help address the mounting challenges such as the shortage of qualified pathologists and the rapidly growing volumes of data. AI and ML are

uniquely positioned to process and gain insight from vast amounts of big data generated across clinical, veterinary, and pharmaceutical pathology.

Many computational pathology methods rely on high quality and consistent metadata, and there is a growing need for explainable AI tools to enable a more widespread adoption of computational pathology. While vendor-neutral data formats such as Digital Pathology and Imaging in Medicine (DICOM) and OME-TIFF would provide a vehicle to capture the relevant metadata, they are not yet widely implemented (Herrmann et al. 2018). Minimum metadata requirements and standards would enable data comparability and quality assurance mechanisms and, alongside the expansion of AI explainability in the field, would maximise the benefits of image analysis, artificial intelligence, and machine learning in Digital Pathology.

3.3.1 IDENTIFIED GAPS IN IMAGE PROCESSING AND ANALYSIS

It has been shown that convolutional neural networks (CNNs) can outperform traditional computer vision methods in many Digital Pathology tasks (Cheerla and Gevaert 2019). They are already being used to help in a clinical environment for routine tasks such as stain quality assessment, cell counting or highlighting slides of interest. However, CNNs rely on appropriate optimisation of network weights and selection of any model hyper parameters, as well as a suitably large training dataset. It is currently unclear, how variations in parameter selection and data affect the output of a CNN, and with it the reproducibility of results. Trustworthy AI-assisted workflows can alleviate the burden on pathologists, help with case prioritisation and enable them to focus on the clinical and diagnostic aspects. The deployment of AI tools, however, requires explainable and trustworthy systems rather than “black box” solutions. A clearer understanding of the parameter space and its effect on model development is needed for reliable progress to be made in the use of AI for Digital Pathology. In particular, determining which methods and visualisations could be used to explain the output of AI/ML models would help the greater community and provide confidence in results. Currently there are no standards in how to visualise pathology data, let alone the results of AI. The inclusion of uncertainties in a pathologically relevant way could improve trustworthiness in Digital Pathology. Moreover, if an algorithm can utilise the uncertainty and variation in the data to provide confidence, or lack of, in predictions this would also increase trust in the Digital Pathology.

Additionally, such methods often require large datasets to train on, but such datasets are not commonly available to the wider research community. The large variation in acquired data due to the physical process of sectioning and mounting the tissue on to a side, as well as the chemical process in the fixation and staining processes. The lack of such datasets mean that it is not possible to compare results on a common standard. We identify that one hindrance to obtaining such a dataset is the cost involved in having a pathologist label training samples, a time-consuming exercise. There is also the caveat that a pathologist’s classification is not perfect, which leads to algorithms training on incorrectly labelled data. Research exists which attempts to combat this using supervised and unsupervised clustering, but there is a lack of guidance as to the value and use of these in this area. An added concern in this scenario is that AI often does not come with a clear use-case guide, and so AI may be applied to systems where the model is not suitable. AI systems should come with clear guidelines of their potential usage scenarios or learn to reject input data when it is not suitable.

Another issue is the linkage of labelling with the pathology images, and subsequent sharing of these. Though the DICOM and OME-TIFF formats are open-source, they are not commonly used for the labelling of data, and so much information about the gathering and formatting of the data is lost or stored in a format that can only be accessed with specific

software. In some cases, these labels can be exported to open formats such as text or XML files, but then these need to be re-linked to the image data introducing potential errors in alignment. Additionally, labels of the data (for classification) are often binary when it is known that most diseases occur on a spectrum, which limits future use. Where grading labels exist, the data are often not sufficiently large enough for ML and are grouped into coarse categories introducing heterogeneity into the classes. In the scenario where a platform captures data, it does so in a bespoke manner which can often limit further analysis to users who have access to a similar system. WSI annotation process is extremely time-consuming, making the annotation data difficult to obtain, nearly impossible to reproduce and therefore extremely valuable. Annotations that are not appropriately linked to the underlying WSI data could be lost or misinterpreted in future use. Introducing data standards for linking annotations with images would facilitate remote annotations of images by multiple pathologists and increase the confidence in the diagnosis and labelling. Additionally, the annotated data could be used to train more junior pathologists and help address the lack of experienced staff.

An additional concern posed by the lack of WSI and annotation data standard is the heterogeneity of clinical systems used in different laboratories. Since clinical computers are often limited with respect to the software they can run, users in different labs use different tools and are often locked-in with a specific vendor. Consequently, much of the metadata is recorded in spreadsheets in inconsistent formats without data validation.

Digital Pathology is becoming more reliant on the use of multiple imaging modalities, where it benefits from the additional biological information about the cells and tissues. However, processing and combination of these non-WSI data and be challenging if the structure and provenance of these new data are poorly understood.
To get the full benefit from novel imaging modalities, multi-level data linkage with WSI data including image registration, sample provenance and processing metadata are vitally important. An example of poor integration brought to attention included integrating “omics” data with WSI data which had been collected at different sites in the organ and thus would not be reflective of the tumour microenvironment. Issues around quality, calibration and uncertainty need to be considered when combining data, and it may not be appropriate to treat all sources equally. The provenance and metadata need to be built into data to enable confident linkage with other modalities. It has been stressed that provenance and linkage-enabling metadata needs to be captured universally, i.e., at the community level, rather than on the laboratory level.

3.3.2 PRIORITISED ISSUES IN IMAGE PROCESSING AND ANALYSIS

The biggest challenge is the ability to transfer pathologists’ annotations on WSI findings between systems and organisations. Seamless transfer of image annotations would vastly reduce the wastage of pathologists’ time, facilitating multiple expert diagnostics including MDT and making the data available for downstream AI analysis. Ideally the annotations should be in a format that easily integrates with multiple systems through import and export functions in the vendor software.

Transparency, trust, and training are key to successful adoption of AI in Digital Pathology. Pathologists need to know why decisions are being made by AI in order to use computerised decision support systems for diagnosis. Standardised outputs and training in how to interpret the results would help bridge this gap.

In both cases vendor buy-in would enable this, though it is unclear if it is better to provide guidance for the vendors to adopt through proprietary import/export functions or encourage a universal system on all platforms. A review of available tools in Digital Pathology as some challenges may have been solved already.

3.4 OMICS AND NOVEL IMAGING MODALITIES

There are a variety of novel imaging and “omics” analysis techniques that could be integrated into a Digital Pathology approach. Many of these methods offer advantages over traditional pathological analysis such as increased diagnostic accuracy, or greater diagnostic information. These methods are not regularly used in clinical practise and this report aims to identify the gaps needed to bring these methods into the clinic and the means to address these gaps.

3.4.1 IDENTIFIED GAPS IN OMICS AND NOVEL IMAGING

There is a need to focus on fresh tissue diagnosis techniques, aiming to progressively rely less on traditional diagnosis techniques such as histopathology. The current understating of which novel techniques are the most useful in each context is poor. There is a need to understand better how these techniques relate to the more traditional and conventionally used ones (i.e.: how their outputs complement and/or corroborate each other). The gradual incorporation of these techniques into the clinical practice relies on a strong interest from the clinicians and other health professionals, who must be aware of them and be able to understand their advantages and limitations. There is therefore a need to cultivate the interest and curiosity of health professionals, especially during their medical school training. Gaining awareness of these techniques at an early stage is likely to potentiate their interest and involvement in the future. There is also a need to improve communication (and ultimately collaboration) between computer scientists and health professionals, which is key to the implementation of these novel techniques in the clinical practise. Simpler and consistent ways to integrate data produced by different instruments or platforms would facilitate their use and effective implementation. Quality assurance would increase the confidence on these novel techniques too.

3.4.2 PRIORITISED ISSUES IN OMICS AND NOVEL IMAGING

Focusing on fresh tissue diagnosis techniques (alongside with their validation and a better understating of their advantages and limitations), as well working on captivating the interest of clinicians and improving the communication between computer scientists and clinicians were considered the most important needs to prioritise in the short term. Improved ways of integrating data and evaluating data quality were considered priorities in a longer term.

3.5 DATA INTEGRATION

Interoperability of pathology data has wide-reaching implications within healthcare, as pathology findings are often used as “gold standard”, e.g. the ground truth that underpins the diagnostic decisions. In clinical setting, pathology reports are required by other diagnostic disciplines such as radiology and are routinely used for multi-disciplinary team meetings (MDTs). This relies upon a seamless data flow between all systems, especially in the context of remote working.

In research, pathology data are used alongside omics and other imaging data in public data resources such as Imaging Data Commons, TCGA, Image Data Resource and many others.

The increasing data volumes and complexity, as well as advances in adoption of Artificial Intelligence/Machine Learning (AI/ML) pose new challenges that need to be addressed as soon as possible to achieve maximum benefit from the advantages brought forth by digitalisation.

3.5.1 IDENTIFIED GAPS IN DATA INTEGRATION

Digital Pathology data is stored in many vendor-proprietary and open formats, depending on laboratory, equipment, and software setup. While vendor-neutral data formats such as DICOM and OME-TIFF exist, they are not widely used due to high development efforts required for adoption. Overall, there is a lack of consensus on minimum metadata content and format that hinders the data exchange between devices, software systems and institutions. The heterogeneity of formats, lack of standards for annotations and metadata present obstacles for deploying AI/ML image processing pipelines.

The metadata required for image interpretation and analysis includes

1. Image-related metadata such as dimensions, colour profiles and compression
2. Patient- and specimen-related metadata, including pathologist reports
3. Sample processing metadata
4. Equipment-related metadata including reviewing and display settings

One of the key issues around the histopathology image metadata arises from the lack of interoperability with laboratory information management system (LIMS) that holds sample processing and patient metadata and cannot be queried by WSI devices. Some vendors provide locked-in WSI/LIMS solutions, but the issue of linking image data with metadata from other systems re-emerges as soon as the image leaves the system, i.e. for analysis, diagnostic review, or image repository. Overall, a variety of image formats coupled with many LIMS solutions make data collection and analysis a difficult exercise that must be repeated every time any of the source data format is changed.

In the context of AI/ML software development, the absence of standards and metadata increases technical efforts to ingest the data or renders the data unsuitable for training due to lack of information about the image and format incompatibilities. This adds to the cost of the product, limits the use of the software, and has regulatory implications when the software needs modification following the image and annotation format changes.

3.5.2 PRIORITISED ISSUES IN DATA INTEGRATION

The key priority is standardisation of the image format and annotations, rather than trying to standardise the entire workflow. A set of consensus standards needs to be developed to enable the flow, exchange and analysis of images and image annotations. These standards should be implemented in a clinically mature vendor-neutral format to enable data collection and exchange.

While it is not possible to include all metadata into the image at the image creation time, we need reliable mechanisms to capture and link the metadata associated with the image. These mechanisms should be able to support high throughput systems and preserve the metadata as images are being exchanged between the institutions. The availability of standardised metadata such as disease or stain type will aid the training, development and operational deployment of AI/ML systems within the Digital Pathology workflow.

The adoption of Digital Pathology coupled with the rollout of AI/ML tools calls for an education program for pathologists. Within the NHS, it would be beneficial to have NHS Digital backed training on data standards, or “standards cookbook” that would educate clinicians and procurement on the availability and suitability of standards in various scenarios. In the UK, the efforts on standards education are undertaken by Informatics committee of Royal College of Pathologists.
Solving interoperability issues between WSI scanners and LIMS, agreeing a vendor-neutral format for annotations and defining the minimum metadata to be stored with the image would enable automated data evaluation and make the whole slide imaging pathway ready for AI/ML deployment, increasing the throughput, reliability and adoption of Digital Pathology.

4 ROLE OF METROLOGY IN DIGITAL PATHOLOGY

Whilst many national and international initiatives to accelerate Digital Pathology adoption and improve its diagnostic reliability are underway, metrology can play a key role in underpinning reproducibility, reducing uncertainty, and providing traceability and comparability at different stages of the diagnostic imaging pathway. As the UK’s National Measurement Laboratory NPL is seen within the sector as an expert in measurement and standardisation. Suggestions on how NPL could help address high-priority issues in Digital Pathology workflow are detailed below.

4.1 SAMPLE PREPARATION AND PROCESSING

NPL’s role in sample processing has been seen in the establishment of standardised quality assurance and quality control (QA QC) procedures for manual and computerised QA processes. Metrology methods could be used to define the metrics to assess these procedures and to ensure sample handling and processing methods are robust and reproducible. These QA QCs can then be used to ensure protocols used at different institutes produce comparable results and add confidence to results produced at contract research organisations and routine pathology laboratories which frequently partner with industry. NPL may also help facilitate a VAMAS study for developed QA QCs and assist the community in instituting proficiency testing.

The implementation of standardised QA QCs and analysis methods should be accompanied by a training programme to contribute to the education of pathologists at all levels as a method of continuing development. Resources could also be tailored to reinforce the importance of the principles of Digital Pathology to future generations of pathologists by providing training to undergraduate and post-graduate students.

4.2 EQUIPMENT CALIBRATION

NPL has an important role in defining and validating equipment calibration materials and standards for different techniques. In particular, NPL can support the development of validated (traceable) calibration methods that improve data consistency and minimise user subjectivity to increase reliability of results and downstream analysis. NPL’s network of collaborators across research, industry and healthcare put it in a strong position to promote adoption of validated methods of calibration within the Digital Pathology community.

4.3 IMAGE PROCESING AND ANALYSIS

It has been recognised that while advanced image processing and analysis tools could help with quality assurance, review prioritisation, diagnosis and reporting, trustworthiness and explainability are key to their adoption in clinical practice. As a national measurement laboratory with the mission to support industry and healthcare, NPL is well placed to provide good practice guides on suitable validation tools, consistent labelling and annotation standards as well as on metrologically sound performance metrics. The latter could help evaluate the uncertainty and fitness-for-purpose of image processing tools, feed into open data and metadata standards as well as help determine the quality and suitability of a dataset for ML-based system training and diagnostic use. Good practice guides on data validation in different laboratory settings, Digital Pathology specific quality metrics and automated reporting have been mentioned as priority work items that would benefit from NPL's input.

4.4 OMICS AND NOVEL IMAGING MODALITIES

Many stakeholders expressed interest in using or are already using multiple novel imaging techniques in their pathology work. To support the use of these techniques in clinical practice, NPL could use its existing expertise in numerous novel imaging modalities including super-resolution and light sheet microscopy, Raman spectroscopy and mass spectrometry imaging. NPL is well placed to support the validation and standardisation of these techniques to ensure confidence in their capabilities and to understand their uses, advantages and limitations in clinical pathology setting. Having established a clear understanding of how these modalities compare and what quality assurance procedures need to be in place to guarantee usable results, NPL can develop training and workshop materials to educate pathologists to help them include the novel imaging tools into their practice.

4.5 DATA INTEGRATION

NPL's expertise in measurement and standardisation could help define the minimum metadata for WSI and annotations and help integrate them within a vendor-neutral information standard. The minimum metadata should be metrologically sound and provide a basis for reproducibility and comparability of Digital Pathology data, increasing confidence in data, analysis and diagnosis and underpinning trustworthy AI.

Once the standards are ready for adoption, NPL's role could encompass concrete tests of interoperability, including benchmarking tests to ensure that solutions work at scale, and recommendations on practical implementations of high-level concepts to make the standards work for end users.

The new standards and the transition to Digital Pathology require training. Together with RCPATH and NHS Digital, NPL should educate pathologists in the use of standards and AI/ML methods. Internationally, NPL's role could ensure the UK developments in Digital Pathology are aligned with the international efforts.

5 FUTURE WORK DIRECTIONS

Digitalisation of pathology offers numerous benefits including improved diagnosis (Bera et al. 2019), new insights into disease phenotypes and mechanisms (Barisoni et al. 2020; Hipp 2020), validation of diagnosis (Snead et al. 2016), and the use of AI to support image quality

assurance and diagnosis. Despite these significant benefits, the adoption of Digital Pathology is limited, and it is currently far away from being embedded in routine clinical workflows. In the workshop on measurement challenges in Digital Pathology that took place in 2021, the participants highlighted an urgent need for data and metadata standards, interoperability, quality control measures, calibration of imaging equipment, trustworthy analysis tools and training of pathologists to understand the benefits and challenges around the digitalisation of pathology laboratories. These needs can be summarised as three key challenges in Digital Pathology; 1) reproducibility within workflows, 2) data interoperability and 3) trustworthiness of data analysis methods.

Metrological solutions can remove these barriers increasing the adoption of technologies and utilisation of digital workflows. The unique placement of NPL as a National Measurement Institute with expertise in metrology for life science imaging and data analysis would allow to translate established metrological concepts such as traceability and uncertainty propagation to support national rollout of Digital Pathology.

5.1 IMPROVING REPRODUCIBILITY WITHIN DIGITAL PATHOLOGY WORKFLOW

Metrology tools can be used to develop good practice for sample collection and appropriate coalition of equipment can reduce variability, increase confidence in the data and improve the accuracy and efficiency of diagnosis. Using our expertise in metrology for life science imaging and building on the existing work, we can identify and control the sources of variation in various stages of the Digital Pathology imaging process, including sample processing and staining as well as technical assessment and quality assurance of imaging equipment and image processing tools. Working with equipment vendors and pathology consortia, NPL can help develop calibration procedures and physical standards to assess the WSI device performance in clinical routine.

Good practice recommendations can then be developed in collaboration with key stakeholders for specific tasks within Digital Pathology, such as diagnosis of a particular disease or stage, quantitative pathology, and integration of conventional H&E imaging with other experimental techniques. Once established, these practices can be disseminated to the community through the engaged stakeholders.

5.2 ESTABLISHING DATA INTEROPERABILITY

Having identified the sources of variation in the Digital Pathology workflow and established good practice, NPL can develop minimum reporting requirements for WSI in clinical and veterinary pathology. These requirements will underpin the interoperable standards for WSI data that will be based on the existing medical imaging standards such as DICOM. Furthermore, the developed good practices and data standards will reduce the barriers for VAMAS studies that can be used to develop quality assurance metrics to facilitate best practice and the formation of new accreditation systems.

A standardised WSI data format will allow capturing data traceability and uncertainty by providing the information on the sources of variation alongside the calibration data to quantify the variations and correct them in subsequent processing. This is particularly important for meaningful aggregation and re-use of WSI datasets in an AI/ML setting. The open data standard in Digital Pathology will enable data interoperability and full utilisation of the opportunities in Digital Pathology through data sharing.

5.3 HELPING BUILD TRUSTWORTHY DATA ANALYSIS TOOLS

The workshop highlighted that pathology data is more than just images and annotations. Patient records, study details, equipment setup and sample processing data are essential for diagnosis, and WSI data should provide means for error-free and efficient linkage to other datasets of clinical relevance as well as a vehicle to add this auxiliary data to WSI metadata if required. This linkage of WSI and non-WSI data sources will increase efficiency in diagnostic and clinical pipelines, reduce the potential for transcription errors and support clinical decision making.

A WSI data standard that (1) provides means to quantify the sources of variation, (2) encapsulates calibration data to correct for these variations and (3) contains sufficient provenance information will enable pathologists to objectively evaluate fitness-for-purpose of the images and promote data re-use. There is a huge potential for re-use for WSI data of known origin and quality in academic, clinical, and commercial settings. The re-use scenarios include virtual biobanks, where images can be used for validation of diagnosis, training of new staff, development of trustworthy interpretable AI systems as well as for the augmentation of WSI data with data from omics and other imaging modalities to gain new insights about the tissue morphology and function.

Acting as a trusted partner, NPL can help establish reference datasets and testing methods to assess WSI data quality and work towards creation of explainable computerised decision support tools including AI and ML. This can be achieved by leveraging our knowledge of AI and ML in a clinical context and by working closely with key stakeholders including NHS Digital, RCPath, BSI and Digital Pathology Innovation Hubs. The stakeholders from all backgrounds have stressed the role NPL has to play in providing the guidance and shaping an appropriate training program to upskill the existing staff and to educate the new generation of pathologists on the role and value of metrology in Digital Pathology.

When combined, the good practices and calibration methods, the WSI data standard that captures uncertainty and traceability, the explainable trustworthy clinically meaningful AI tools and the appropriate training will upskill pathologists in AI and ultimately improve the adoption of Digital Pathology that is underpinned by metrology. Overall, NPL's involvement in Digital Pathology would increase the confidence in data and analysis tools, reduce the potential for errors, decrease the time-to-diagnosis, and improve quality of life.

6 SURVEY RESULTS

The pre-workshop online survey took place between November 2020 and January 2021. The aim of the survey was to enhance the literature review findings and to shape the group discussions during the workshop. The survey included 31 questions in the following categories:

- 6 questions related to the background of respondents and their specialty areas
- 10 questions related to the acquisition of imaging data, storage, lookup and integration of such data with other systems
- 5 questions related to quality assurance and other relevant regulations
- 4 questions related to artificial intelligence and machine learning in Digital Pathology
- 3 questions related to the future directions of Digital Pathology
- 3 concluding questions about prioritisation of work and other challenges

6.1 INFORMATION ABOUT SURVEY PARTICIPANTS

The twenty-four respondents that took part in the survey identified their organisation category as either Industry, Academia, NHS or Other. The majority of responses were from industry (N=12), including WSI and other equipment vendors (N=3), software vendors (N=4) and pharmaceutical companies (N=5), followed by academia (N=6) and clinical pathologists working in various NHS hospitals (N=5). Most laboratories covered by the survey used multiple modalities (Figure 4), with whole slide imaging being the most common (N=20), followed by immunohistochemistry (N=14) and then immunofluorescence multiplex assays (N=11). Of the respondants which came from laboratories that processed slides, the majority came from laboratories that processed over 5000 slides per year.

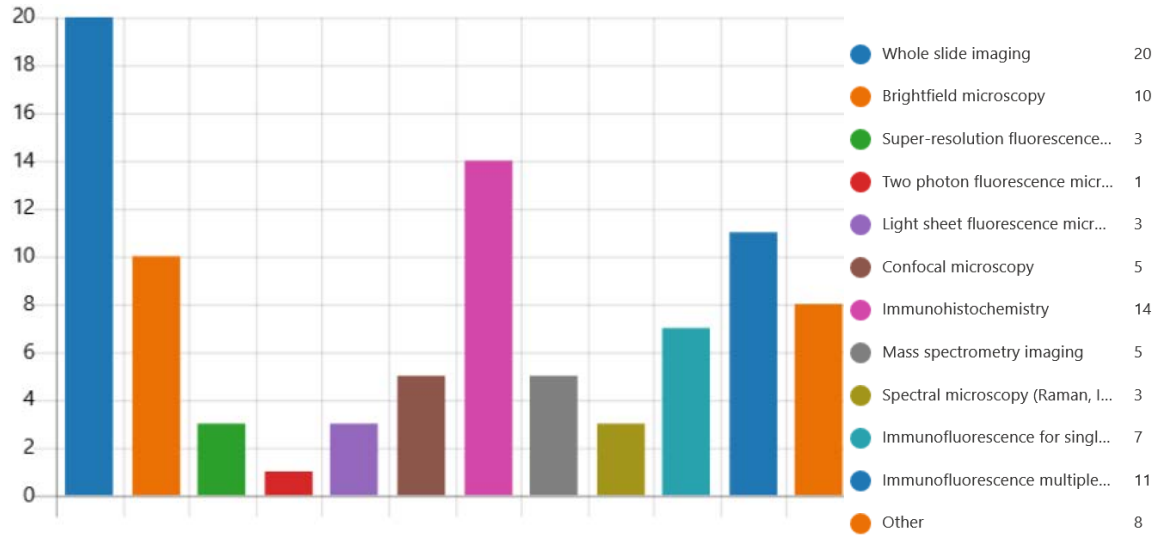

Figure 4: Imaging modalities in use in the laboratories of the participants

6.2 USE OF HISTOPATHOLOGY IMAGING DATA

The Digital Pathology workflow is complex and requires specialised software at different stages. The software should be able to work with relatively large images, as the average image size per slide is between 1 and 10 GB. Figure 5**Error! Reference source not found.** shows what type of software respondents tend to use at each stage to process these images. It appears that commercial software is often preferred, apart from when the issue is image registration, where software developed in-house was the most used tool, suggesting more specific requirements for this domain that might currently not be met.

Over 70% of respondents shared Digital Pathology samples as identified in **Error! Reference source not found.** with other organisations, and of these most shared multiple types of samples. Participants shared data for many different reasons, with no clear standout. For example, images were shared for the purposes of gathering or giving a second opinion, to assist with data requirements or to support drug discovery. As a likely consequence of this sharing network many respondents stated that their data was either stored on a networked computer or in cloud storage, with only a minority storing slides on an unnetworked computer. Many participants also have archiving procedures implemented for their data needs.

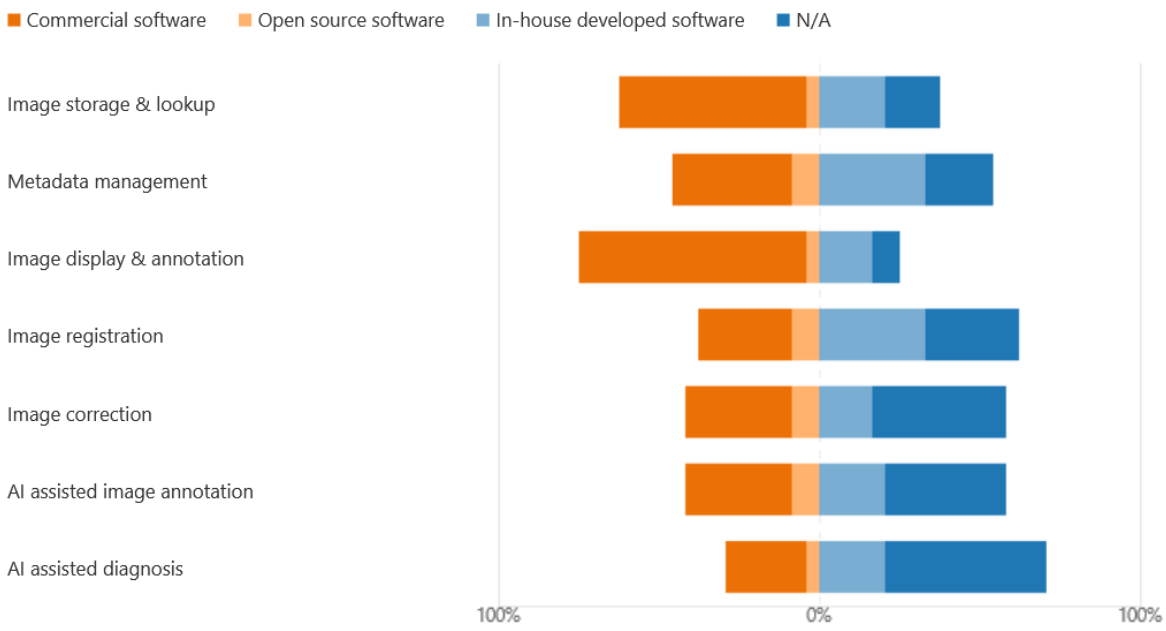

Figure 5: Use of commercial, open source or in-house software across the survey participants.

Interestingly, respondents appeared to be able to find data suited to their needs with only a little effort, rating it an average of 3.83/5.00 for ease (minimal value possible being 1). However, they were more neutral about the ease of combining image data with other relevant data used for diagnostic work ranking it a 3.00/5.00. This question did receive a somewhat polarised response, as though 50% of the respondents selected 3.00, 20% selected 1.00 and another 20% selected 5.00. Other relevant data included patient records, study protocols, laboratory results, 'omics' data and radiology images as additional data they used in their work. When prompted for additional information, some of the challenges faced by participants included:

- The fact that the data to combine was on different systems (sometimes with no interconnectivity)
- Metadata curation to ensure that the necessary information was recorded and well annotated
- Compatibility between vendor specific formats and data retention
- Registration of images

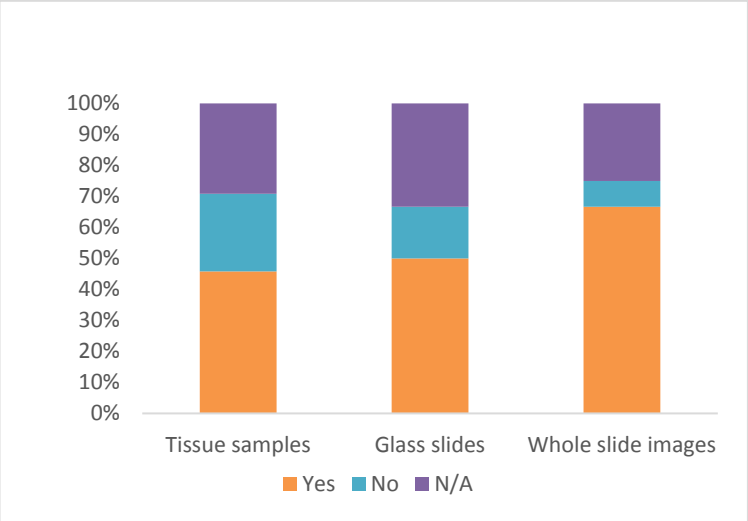

Figure 6: Percentages of respondents who exchange tissue samples, glass slides or images with other organisations

File formats were an important topic of conversation during the workshop, and that was reflected in this questionnaire, as the majority of respondents typically use vendor-proprietary formats for their work. This is followed by generic image formats such as TIFF and JPEG, which can only hold limited meta-information. More sophisticated formats such as DICOM for pathology and OME-TIFF do not appear to be as regularly used.

6.3 QUALITY ASSURANCE

Over 70% of respondents came from workplaces where good laboratory practice applied. Of these, over 75% also applied good clinical practice. Some respondents also came from workplaces that implemented current good manufacturing practice, relevant ISOs and software-specific practices. Quality assurance protocols were regularly applied to tissue management equipment, imaging equipment and the image analysis software/pipeline. There was an even interest in calibration tools related to uniformity of illumination within the field, magnification, and colour calibration.
Protocol for calibration of the equipment used can vary significantly by respondent, with a small percentage stating they have daily external QA by an equipment vendor as may be seen in Figure 7 (which is clarified to be an automatic routine implemented in the equipment). More commonly, vendors will carry out bi-yearly checks, though a third of responders state that QA by the vendor was only carried out upon installation. Independent assessors are mostly used upon installation, or bi-yearly. Internal QA can be more varied. It appears that approximately 45% of the equipment used was only calibrated once internally upon installation, 20% of the equipment is calibrated monthly, ~16% bi-yearly and the remainder

either weekly or daily. Many participants (N=14) did not however know the details around QA procedures, so these must be seen as best guesses.

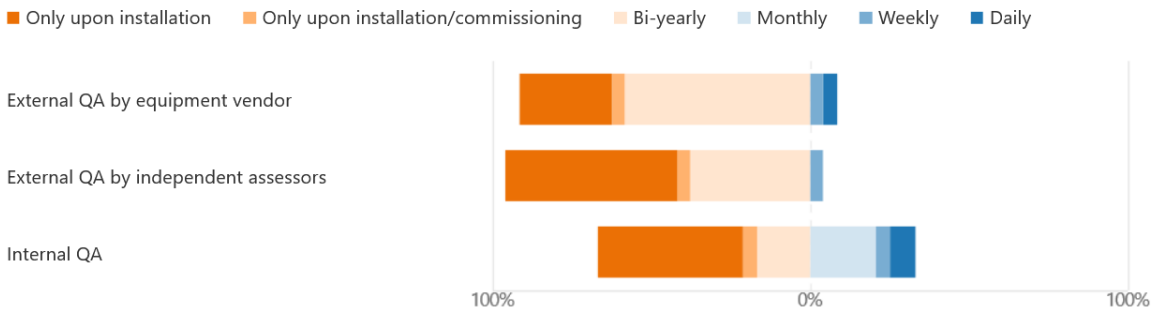

Figure 7: Frequency of equipment calibration.

6.4 ARTIFICIAL INTELLIGENCE & MACHINE LEARNING

The survey results reflect the growing interest in the use of AI and ML methods in Digital Pathology. In particular, the results indicate that these methods are being used mostly for cell type annotation and disease classification (Figure 8). Additionally, many people expressed an interest in using such methods for the removal of poor quality images from datasets, image registration, image lookup and navigation. Only few people stated an outright disinterest in specific AI applications. At the same time, the respondents were less confident to use AI or ML in some use cases, perhaps due to a lack of familiarity outside of their area of expertise. Where confidence was stated, the dominant sentiments were that it works well some or most of the time. In order to increase confidence in AI, many participants stated that they would like an improved transparency of the decision making process, the validation of algorithms by an independent party and the benchmarking of software on independent datasets (Figure 9). Currently, it appears that the performance of such tools is assessed qualitatively using individual pathologists' experience, with some laboratories attempting to minimise bias by introducing multiple human reviews and pre-arranging thresholds for use.

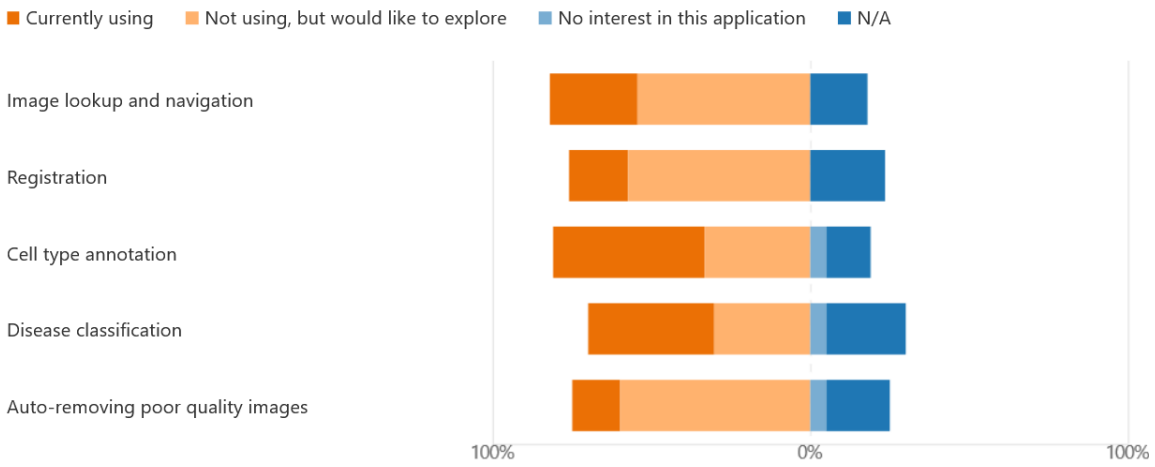

Figure 8: Areas of application of AI/ML methods in digital pathoogy grouped by participants' interest and usage.

6.5 FUTURE DIRECTIONS

Many respondents expressed interest in adding further imaging techniques to their repertoire, with genomic and targeted proteomic imaging being the most desired modalities. Among other imaging techniques that were of interest to the participants, the survey highlighted the following:

- Liquid biopsy techniques (Palmirotta et al. 2018)
- Radiomics
- Combinations of metabolite and multiplexed imaging
- Whole animal imaging
- Spatial transcriptomics
- Fast light-microscopic analysis of antibody-stained whole organs
- AR and VR with 3D holographic WSI reconstruction
- Mass spectroscopy

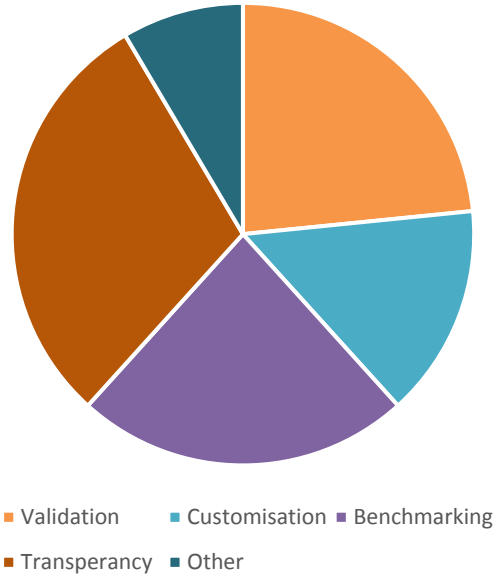

Figure 9: Summary of techniques and tools that would improve respondents' confidence in AI/ML usage in digital pathology.

There was also a widespread interest in software tools to combine data from multiple modalities with H&E images.

6.6 PRIORITISATION OF WORK

Participants of the survey had some clear priorities as may be seen in Figure 10. The responders highlight an urgent need to address WSI image processing using AI and machine learning. There is also some demand for the integration of WSI with other

data and for improved WSI data lookup and navigation. Some priorities that were also highlighted were improved data sharing, uncertainty estimation and standards.

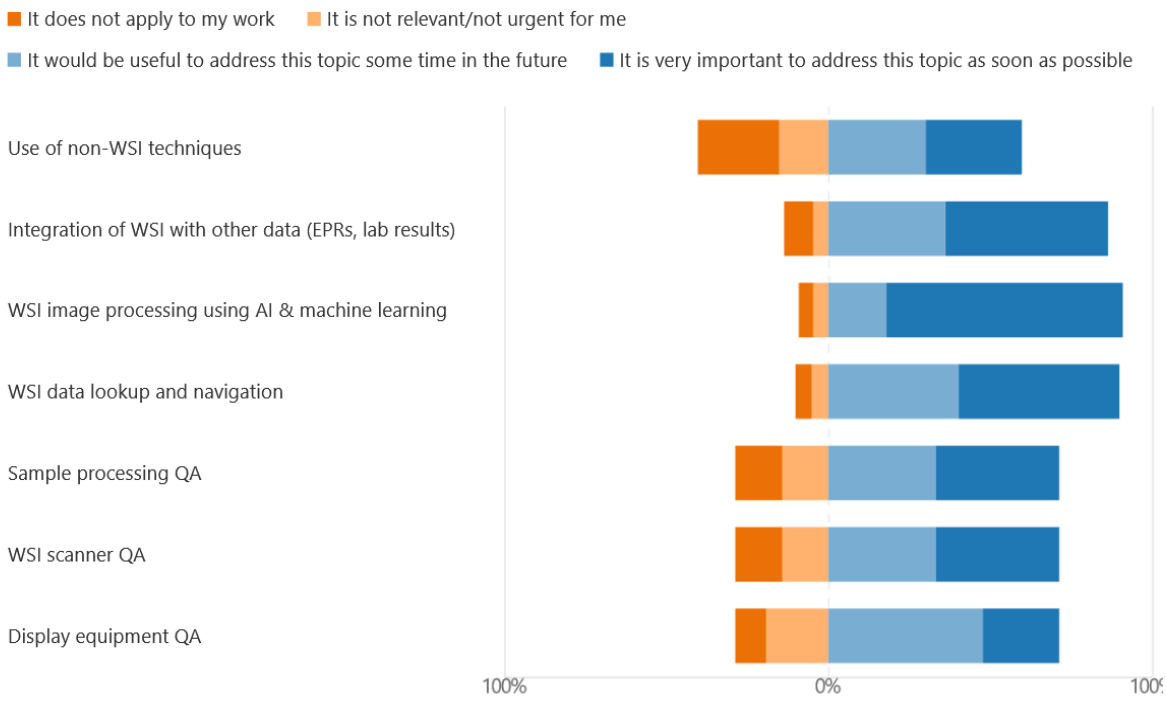

Figure 10: Digital pathology workflow components ordered by the level of relevance by the survey participants.

7 WORKSHOP PARTICIPANTS

Table 1: Metrology in Digital Pathology 2021 workshop participants and their allocation to discussion groups.

Organisation name	Organisation type	Discussion groups
Ashford and St Peter's Hospitals NHS Foundation Trust	NHS	Equipment Calibration
AstraZeneca	Industry (pharmaceutical)	Image Analysis, Novel Imaging, Sample Processing
British Standards Institution	Standards bodies	Data Integration
Cambridge University Hospitals	NHS	Sample Processing
Charles River Laboratories	Industry (pharmaceutical)	Image Analysis
DesAcc	Industry (software)	Data Integration
DICOM	Standards bodies	Data Integration
FFEI Ltd	Industry (equipment)	Equipment Calibration
GE Healthcare	Industry (software)	Image Analysis
GlaxoSmithKline	Industry (pharmaceutical)	Data Integration, Equipment Calibration, Sample Processing
IHE	Standards bodies	Data Integration
Leeds Teaching Hospitals NHS Trust	NHS	Equipment Calibration
Leica Biosystems	Industry (equipment)	Image Analysis
NHS Digital	NHS	Novel imaging
Nikon Corporation	Industry (equipment)	Equipment Calibration
Norfolk and Norwich University Hospitals NHS Foundation Trust	NHS	Data Integration
Paige.AI	Industry (software)	Data Integration, Image Analysis
Royal Brompton and Harefield Hospitals	NHS	Sample Processing
Royal Surrey County Hospital	NHS	Equipment Calibration
Royal Veterinary College	Academia	Sample Processing
Smith + Nephew	Industry (equipment)	Sample Processing
Surrey Heartlands	NHS	Novel Imaging
University College London	Academia	Image Analysis
University of Cambridge	Academia	Image Analysis, Equipment Calibration
University of Oslo	Academia	Image Analysis
University of Surrey	Academia	Image Analysis
Zeiss	Industry (equipment)	Equipment Calibration

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