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Poster Presentation Abstracts

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Contributing Author(s)	Hitesh Dave, Steven Taylor, Deon Hildebrand
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Poster Title	Digital Image analysis in GSK (- a useful tool for safety, efficacy and animal modelling)
Abstract	Digital Image analysis provides extraction of meaningful data from digital images through computer software derived algorithms. Essentially it is the quantitative characterisation of features within digital images. Image analysis adds value in the discovery of medicines demonstrating: 1) Objective quantitative data comparing normal with diseased tissues, including; histological/pathological features, gene expression - ISH amplified/labelled message and IHC labelled protein 2) Quantification of safety and efficacy endpoints, 3) Quantification of end points required in the validation of animal models of human disease, all of these data sets are used to generate statistical results and graphs that are used to facilitate evidence-based decisions.

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Poster Title	Validation of Whole Slide Imaging (WSI) for primary surgical pathology diagnosis of prostate core biopsies
Abstract	**Objectives**: To assess the concordance of surgical pathology diagnosis on WSI with conventional glass slide (CGS) diagnosis and to compare the time spent during both processes. **Methods**: The glass slides of already reported prostate core biopsies during the period January 2016 to December 2016 representing various benign and malignant pathologic diagnoses and WSI of the same slides were reviewed by 2 pathologists independently, with a washout period of 1 month. All slides were digitized using Pannoramic MIDI II scanner by 3DHistech, Budapest, Hungary. Concordance between both diagnoses and time spent during both processes were assessed. IBM SPSS version 22.0 was used for calculating the kappa statistics (κ). Minor and major discrepancies were analysed. **Results**: Total 60 cases were studied. Intra-observer agreement for diagnosis between WSI and CGS was 96.3% (κ = 0.9; 95% confidence interval [95% CI]: 0.8–1 for observer 1, and 96.4%, κ = 0.9; 95%CI: 0.8-1 for observer 2). The intra-observer agreement between the two processes for the Gleason scores assigned was substantial (75.6%, κ = 0.7; 95% CI: 0.6–0.8 for observer 1, and 72.5%, κ = 0.7; 95%CI: 0.6-0.8 for observer 2). The average time taken to arrive at a diagnosis using CSG and WSI respectively was 40sec & 34sec for observer 1, and 72sec & 71sec for observer 2. There was one major discrepancy each with both the observers wherein the diagnosis was altered. Minor discrepancies reflecting the change in the Gleason scores were seen in 4 and 2 cases with observer 1 and 2 respectively. **Conclusion**: WSI is comparable to CGS for diagnostic surgical pathology. Diagnosis of prostate core biopsies using WSI was accurate. The WSI can prove useful adjunct in routine diagnostic pathology reporting.

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Poster Title	Identification and classification of dysplastic colon polyps using image recognition techniques
Abstract	Colorectal cancer is a major health problem. Early diagnosis can be established by a systematic endoscopic screening of the whole target population. The scale of the systematic screening increases the burden on pathology laboratories, especially in combination with the limited available time frame to process the samples. Whole slide scanners can provide whole slide scans (WSI) of classical glass slides. The WSI can be analyzed manually by a pathologist or automatically by using “Digital image recognition techniques”. These techniques have been improved greatly thanks to advances in deep learning and “Artificial Neural Networks” can be trained to recognize patterns in digital images with high accuracy. The aim of the study is to design a model, based on machine learning techniques, that recognizes major zones of interest: normal tissue, low grade- and high grade dysplasia and invasive carcinoma on WSI of colon biopsies. In a pilot study we were able to build a model identifying abnormalities with an accuracy of 95% based on 190 digital images. In a follow up study we examined 73 WSI of colonic polyps made by a Philips scanner. Annotation of zones of interest were made independently by 4 gastro-intestinal pathologists. Based on these data we designed and trained a machine learning (ML) model that recognizes and classifies accurately zones of interest focusing on pattern recognition and not on object (cell) detection/classification. This model can be used as a pre diagnostic tool that offers support to the pathologist and will help to provide an accurate pathology report within a short time.

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Poster Title	Identifying and staging breast cancer metastases in lymph nodes using HALO-AI, a neural network integrated into the HALO image analysis platform
Abstract	The Camelyon17 challenge (https://camelyon17.grand-challenge.org/) was organized by Diagnostic Image Analysis Group (DIAG) and Department of Pathology of the Radboud University Medical Center [1]. The purpose of this challenge was to come up with a fully automated method to find breast cancer metastases in whole slide lymph node images and classify each lymph node into one of four stages: 1) negative, 2) isolated tumour cells (ITC), 3) micrometastases, or 4) macrometastases, and combine these results across five sections per patient to give a tumour level stage, pN0, pN0i+, pN1, or pN2. Here, we describe the use of a VGG-style neural network [6, 7] integrated into the HALO platform (HALO-AITM) to detect metastatic breast cancer cells in the Camelyon17 challenge. The neural network was trained on whole slide lymph node images including ground truth annotations provided by the Challenge. The final classifier was tested on 500 lymph node images (5 slides x 100 patients). The Indica Labs submission achieved a weighted kappa score of 0.8666 and was the top ranking commercial solution submitted prior to the challenge deadline.

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Poster Title	CM-Path: Re-Invigorating Cellular Molecular Pathology in the UK
Abstract	The NCRI is a Charitable Incorporated Organisation, a UK-based partnership of cancer research funders working together to accelerate research progress for patient and public benefit. In 2016 the NCRI set up an initiative called Cellular Molecular Pathology (CM-Path) which is a five-year venture with the aim of reinvigorating academic pathology in the UK. In the era of personalised medicine there is a rapidly escalating need for innovative testing to assess prognosis. However, over the past 15 years the pathology workforce has been substantially eroded at all grades from lecturer to professor. CM-Path focusses its activity in four main areas to reverse this decline in academic pathology: Skills and capacity – training and upskilling of pathologists at all levels of the workforce Clinical trials – ensuring that expertise is available to support clinical trials which are dependent on pathological analysis. Highlighting the need for pathology involvement up front in clinical trials. Discovery – facilitating best practice relating to tissue handling, biomarker research and tissue access Technology and informatics – supporting pathologists to gain expertise in, evaluate and deliver appropriate novel tissue based diagnostic tests including digital pathology CM-Path has been running for almost three years now, over this time we have achieved many things to promote and raise awareness of the importance of pathology research including publications in high impact journals, engaging and educational workshops, creation of advisory groups, forums for discussions and tools to enhance best practice tissue quality. More information about rh CM-Path programme can be found on our website here: https://cmpath.ncri.org.uk/

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Poster Title	Integrated analysis of digital pathology, multi-omic and biomarker data for understanding the tumor microenvironment in precision immuno-oncology trials
Abstract	**Introduction** Digital pathology in conjunction with multi-omic and biomarker data allows for the detailed assessment of patients' tumor microenvironment in clinical and translational research. The data can help predict whether the patient will likely benefit from immuno-oncology therapies and therefore improve treatment outcome. There are several challenges associated with the integration & analysis of digital pathology, multi-omic & biomarker data: • Data is often siloed and spread across different locations within organizations which makes data access complex, time consuming, and error prone • Data exchange capabilities and interoperability of different data readouts from various software platforms are limited and do not allow for comprehensive data/result utilization • Chain-of-custody for complex, multi-step digital pathology workflows is often difficult to provide due to a lack of standardization • Current data reporting processes to regulatory authorities requires usually manual error prone data convergence steps • Increasing amount of data and users create challenges with respect to scalability and performance of data analysis and management solutions **Methods & Results** Genedata Profiler is an enterprise software platform for translational and clinical research that provides core capabilities addressing all challenges mentioned above: • Integration of digital pathology, multi-omic and biomarker data from all locations across your organization to centrally manage those data across multiple studies supported by a secure, study-centric data management system • Standardized vendor-agnostic image processing, viewing, and sharing capabilities • Data governance and complete chain-of-custody for digital pathology workflows • Automated regulatory submission data preparation, e.g. CDISC SDTM format • Efficient and scalable operation in cloud and HPC environments **Conclusions** Genedata Profiler provides a validated and scalable enterprise software platform for managing and processing of digital pathology data, multi-omic, and biomarker readouts for translational and clinical research. The platform enables translational and clinical research organizations to achieve their vision of precision immuno-oncology by leveraging the full power of integrating digital pathology, multi-omic and biomarker data.

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Poster Title	Evaluation of the stroma - a necessary parameter in the effective diagnosis of cancers. Example of papillary thyroid carcinoma
Abstract	Recognition of true stroma consistency is necessary to diagnose the right cancer prognosis. In stromal functionality, like in tumor nature, age plays an important role. One of the aging mechanisms is genetic material damage. Damaged cells acquire paracrine features resulting in various secreta (proinflammatory, stimulating growth or angiogenesis, etc.) favoring the development of malignant tumors. The stroma-cell damage phenomena are not included in the routine histopathological diagnostics and pathologists' knowledge is limited in this respect. In my research, I used DNA damage markers (53BP1,ɣH2A.X) to asses papillary thyroid cancer (PTC) and non-invasive thyroid neoplasm with papillary-like nuclear features (NIFTP) stroma. I aimed at showing a correlation between the damaged stroma cells and the type of tumor. PTC constitutes a significant diagnostic problem. For many years, cell nuclei images were considered the key morphological parameter of PTC. Within the last few years, NIFTP were distinguished and included in the WHO classification (2017). That allows for avoiding onerous treatment in a large percentage of cases, but simultaneously makes PTC diagnostics more complicated in terms of qualifying patients to the risk group. The research results determine the difference between the number of damaged fibroblasts in the PTC stroma and the stroma of NIFTP. Expression of 53BP1 and ɣH2A.X was significantly larger in the case of PTC and there was a connection between malignancy and stroma consistency. Moreover, in the case of PTC there was strong statistical interdependence between DNA damage markers expression and the summary dose of I131 in iodine therapy after thyroidectomy during 5 years observation, which suggests that interpretation of stroma condition may be required in cancer prognosis.

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Poster Title	Multiplex immunohistochemistry using SIMPLE staining method and digital pathology
Abstract	More and more molecular markers emerge in a variety of fields encompassing cancer and inflammatory diseases. Visualization of different markers on the same slide is strongly demanded to clarify the complicate interaction between different types of cells. Traditional chromogen immunohistochemistry confines number of labels available and is weak at colocalization within a single cellular compartment. Although multicolor immunofluorescence (IF) could detect more than three antigens, it always needs to overcome autofluorescence. In addition, the stained slides with multiplex IF remain accessible for a short period of time. Recently, sequential immunoperoxidase labeling and erasing (SIMPLE) method was developed. Combined with digital scanning, it enables localization of multiple antigens in cells and tissue with primary antibodies from the same species. This novel protocol has been utilized in a limited number of reports so far. In this study, we used Hamamatu photonics nanozoomer and the digital pathology free application software, Qupath. Staining time, staining substrate, and scanning method were tested and optimized. The application of the multiplex immunohistochemistry will be demonstrated in lung cancer and interstitial pneumonia. Finally, the pros and cons of the technique will be discussed.

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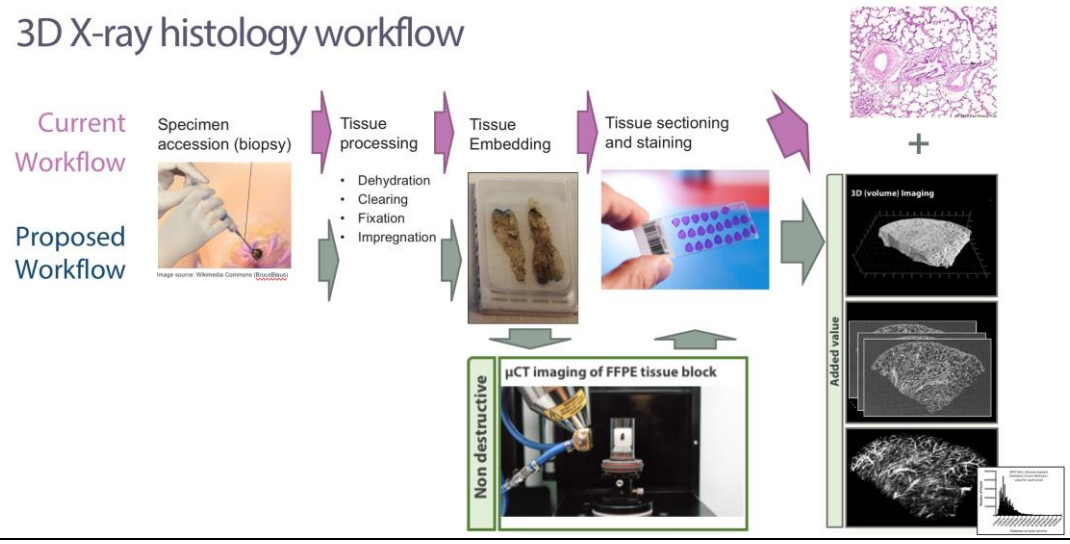
Poster Title	3D X-ray histology by means of micro- Computed Tomography
Abstract	Living structures are an intricate three-dimensional (3D) arrangement of cells and tissue matrix across many length scales, yet conventional histology is constrained in two dimensions (2D). X-ray micro-computed tomography (µCT) allows for non-destructive 3D (volume) imaging of the microstructure of specimens, but has historically been considered unsuitable for histological analysis of soft tissue biopsies due to the limited X-ray contrast between soft tissues and the embedding medium (e.g., wax). However, we recently demonstrated that µCT can successfully resolve microstructural detail [1] of routinely prepared tissue specimens, to a degree that can overturn previous erroneous understanding of disease pathogenesis [2]. Here we introduce '*3D X-ray histology*': a non-destructive µCT-based imaging approach, capable of complementing conventional histology with truly 3D microstructural data of standard non-stained, formalin-fixed and paraffin-embedded soft tissue biopsies (cf. Figure). The technique relies on optimised µCT imaging protocols developed in-house using the first-of-kind µCT scanner for high-resolution imaging of low-contrast tissue specimens (Nikon, Med-X prototype). First results show consistent and reproducible image quality characteristics at (isotropic) voxel sizes that range from 4-10 µm, enabling qualitative inspection and quantitative image-based characterisation of the tissue. We also demonstrate how combining µCT and 2D convectional histology can add "specificity" to the technique permitting localisation histological features like 3D networks and individual cell types for quantitative volumetric image analysis; e.g. spatial distribution of specific cell-types in the tissue and relation/proximity to blood vessels and airways. 3D X-ray histology can be readily applied to a plethora of archival materials and routinely-processed tissue samples, yielding unprecedented opportunities for data mining via digitising archival tissue. Importantly, the non-destructive nature of the technique means that it can be directly integrated into existing histology workflows. This could enrich conventional 2D histology with capabilities including close-to-native and whole biopsy imaging, any-orientation virtual sectioning or assessment of 3D structural features such as volume, connectivity and heterogeneity. [1] AE Scott, et al., PLoS One 10.6 (2015): e0126230; [2] MG Jones, et al., JCI insight, 1.5 (2016) 

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Poster Title	Large scale consensus when scoring randomized sets of digital pathology slides: study design
Abstract	Introduction The incidence of ductal carcinoma in situ of the breast (DCIS), a precursor of invasive breast cancer, has greatly increased due to population-based breast cancer screening. Our aim was to explore the value and robustness of pathological findings of DCIS by evaluating interobserver agreement in relation to clinical outcome. To this end we have explored the use of digital slides and online collection of answers from 56 participants from 18 European countries. Material and methods 353 cases of pure DCIS from a nation-wide cohort diagnosed between 1993 and 2004 were selected for analysis. Tissue blocks were collected from the DCIS lesions. One representative slide was scanned at 40x magnification for each lesion. This set was split into 100 slides to be scored by everyone and 253 slides to be scored by 10-11 participants. Each pathologist was assigned the common 100 slides and a unique set of 46 slides in random order to ensure uniform distribution of scored slides even if not all participants finish scoring. An in-house developed slide viewer (Slide Score) was utilized to collect responses in a standardized way and store them in a database that will allow to give each pathologist personalized feedback. The following pathologic variables were scored by 54 European pathologists and 2 pathology trainees on digitized whole slides: presence of DCIS, dominant growth pattern, grade, mitotic rate, calcifications, necrosis, stromal and inflammatory response. Interobserver agreement will be analysed and related to clinical outcome. Results and discussion By publication date all but 1 slide have been scored, 244 slides have been scored by at least 3 participants, 157 by at least 5 and 50 by at least 22 participants. 18 participants have scored all their assigned slides. Conclusion Randomized assignment of scoring set facilitated uniform distribution of scores. Interobserver agreement classifying DCIS will determine which pathologic variables can be robustly used for reliable risk stratification.

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Poster Title	Optimization and image analysis of RNAscope® technology on 3D human organotypic ciliated respiratory epithelial culture.
Abstract	**Introduction**: Advanced Cell Diagnostics' (ACD) RNAscope® in situ hybridization (ISH) technology is used to visualize and detect RNA of interest in a cell-specific manner on formalin-fixed paraffin-embedded tissue sections. RNAscope® technology is a good alternative to immunohistochemistry, the success of which is strictly dependent on antibody availability. The objective of this work was to optimize the RNAscope® method for human organotypic nasal cultures and quantify the staining using Definiens software complemented by a fit-for-purpose, custom-built plug-in developed by Definiens. A quantitative assessment is necessary to evaluate an optimized RNAscope® protocol. **Material and methods**: Organotypic nasal cell cultures were fixed, processed, embedded, cut, and stained with hematoxylin-eosin and Alcian blue for morphology assessment. The unstained cut slides were then processed for RNAscope® optimization using the Leica Bond Rx instrument. Three crucial parameters (heat pre-treatment, enzymatic digestion, and signal amplification) were tested and optimized. The slides were scanned using the Hamamatsu NanoZoomer 2.0 slide scanner. Regions of interest were automatically detected by the custom-built plug-in, which identifies tissues and excludes membranes. Image analysis was then performed using three main steps. The nuclei were detected based on hematoxylin color intensity threshold and their average size, cells were simulated by Definiens software, and stained RNAscope® spots were detected by color intensity and size of dots. **Results**: The optimized RNAscope® protocol showed robust staining on a positive control probe and no staining on a negative control probe. The quantification results presented an average quantification score of 3 for the positive control probe and 0 for the negative control probe. According to ACD recommendations, 0 is the lowest score, and 4 is the highest score. The image analysis confirmed the optimization of the RNAscope® technique for organotypic nasal cultures. **Conclusion**: The quantification results demonstrated an accurate measurement of the optimized conditions for ISH using the RNAscope® technology on organotypic cell culture samples. It was also observed that qualitative assessment alone is not sufficient for RNAscope® analysis.

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Poster Title	Development of a software for pathological image diagnosis using Deep Learning.
Abstract	Convolutional neural network (CNN) is effective in image recognition. According to Microsoft research in 2015, CNN classified images with higher accuracy than humans. CNN classification is also useful in pathological image diagnosis. The proposal of this research aims to put pathological AI diagnosis into practical use. We constructed pathological AI diagnosis service, "PidPort" that supports learning of multiple organs. We are using a SuperComputer for image learning, that is why our AI learns data very quickly. This works on web, so it does not require the user's PC's performance. We got WSI (whole slide images) data from several faculties; Kyushu university and Hiroshima university, and so on. We applied rotation of images, Image segmentation and Transfer Learning. Transfer Learning is the reuse of a pre-trained model on a new problem. It is currently very popular in the field of Deep Learning because it enables you to train Deep Neural Networks with comparatively little data. This neural network is based on inception V3. The unnecessary region is removed from the WSI, and the interest area ROI is cut out. The ROI is divided into small size images (299 dots*299 dots). We classified these stomach small size images into 5 classes; adenocarcinoma, adenoma, gastritis, normal, others. The classification accuracy was 97.5%. Clinical trial started at multiple facilities. We will continue to improve accuracy by acquiring new data at the clinical site. The number of pathologists is not enough. "PidPort" can help pathologists and reduce misdiagnosis.

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Poster Title	Getting a broader picture – the benefit of digital slides in the simultaneous visualization of short and long RNAs and proteins
Abstract	The techniques of visualizing several molecules simultaneously in tissue sections has developed tremendously over the last past years. The need of understanding the complexity of the cellular context of cancer tissues, e.g. the presence of inflammatory cells in connection with novel immuno-oncology therapies has contributed to accelerate the technologic development. However, the technical challenges are many, both in the development of staining instruments that handle multiple staining procedures and in the development of slide scanners that handle multiple fluorescence signals and large image files. MicroRNAs are short pieces of single stranded RNA that are known to bind mRNA to regulate the translation output. Thereby microRNAs contribute to control cellular protein levels. Because of the presence of these essential molecular networks, it is appealing to visualize microRNA, mRNA and protein in the same cellular context. We have developed an automated multiplex fluorescence approach for the analysis of inflammation in colon cancer tissues. As a technical example, we combined staining of interleukin-1□ mRNA, cytokeratin and microRNA-17. The combined staining of the three molecular forms with three different detection methods, LNA-based ISH, immunohistochemistry and RNAscope revealed that interleukin-1□ mRNA is upregulated in inflammatory cells in focal areas of cancer cell de-differentiation and invasion and that microRNA-17 expression is lost in the de-differentiated cancer cells. Visualization of the fluorophores and slide scanning was performed using 3D-HisTech's Pannoramic scanner, which has the advantage of acquiring images at multiple confocal layers that can be examined separately.

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Poster Title	Utilization of fluorescent multiplex IHC and digital image analysis for studying LAG-3, CD3 and CD8 positive TIL subsets in NSCLC tissue
Abstract	Targeting checkpoint molecules expressed on immune cells has shown promising results in the treatment of non-small cell lung cancer (NSCLC). One interesting molecule that is frequently expressed on exhausted tumor-infiltrating lymphocytes (TIL) is lymphocyte activation gene-3 (LAG-3). We recently implemented chromogenic anti-LAG-3/CD3 dual immunohistochemistry (IHC) and digital image analysis to quantify LAG-3 positive T cells in NSCLC tissue. Here, we extended this work by establishing an anti-LAG 3/CD3/CD8/pan-Cytokeratin (pan-CK) fluorescent multiplex IHC (mIHC) assay followed by digital image analysis to examine the composition of T cells infiltrating NSCLC tissue in more detail. Tyramide-signal amplification (TSA) based fluorescent 5-color mIHC (LAG 3/CD3/CD8/pan CK + DAPI) was developed by Indivumed on the Leica BOND RX automated staining platform and applied to formalin-fixed paraffin embedded (FFPE) NSCLC tissue samples. Image analysis was performed by OracleBio. Tumor and stroma regions of interest (ROI) were classified per core according to the pan-CK and DAPI signals. LAG-3, CD3 and CD8 single positive cells, as well as dual and triple positive cells, were then quantified in the tumor and stroma ROIs. Fluorescent mIHC allowed for a specific quantification of LAG 3, CD3 and CD8 single, LAG 3/CD3, LAG 3/CD8, CD3/CD8 dual and LAG 3/CD3/CD8 triple labeled cells in the tumor and stroma ROIs of the analyzed NSCLC samples. As part of the assay validation, similar ratios of the differently labeled cells, in particular LAG-3 positive T cells (LAG-3/CD3 dual positive), were observed with fluorescent mIHC and chromogenic IHC, demonstrating a good concordance between the two approaches. Among the analyzed NSCLC samples, different ratios of LAG 3 positive TILs in adeno- and non-adenocarcinoma samples were detected. Determining the ratios of dual or triple positive TIL subsets by mIHC in combination with digital image analysis allows for a clearer understanding of the lymphocyte composition within NSCLC tissue than a simple quantification of LAG-3, CD3 and CD8 single positive cells. Furthermore, such information will contribute to a deeper understanding of the role of LAG-3 positive immune cell sub-populations in the progression and treatment of NSCLC cancer.

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Poster Title	Classifying Leukocyte Morphology with Deep Convolutional Neural Networks
Abstract	Examination of Leukocyte cytomorphology using light microscopy, a method dating back to the nineteenth century, remains an important cornerstone in present-day Leukemia diagnostics. In contrast to other laboratory tests, cytomorphological examination has so far defied automation and to this day is usually performed by trained human examiners. Hence, the diagnostic yield of that method is highly operator-dependent and hard to correlate quantitatively with other diagnostic modalities or clinical data. We digitised a set of 100 blood smears without pathological findings and 100 blood smears taken from patients with different stages of Acute Myeloid Leukemia (AML) from the University Hospital of Munich Laboratory for Leukemia Diagnostics during 2014-2017. All blood smears come from routine diagnostics, were stained using Pappenheim‘s stain and scanned at 100-fold magnification and oil immersion with a digital microscope - scanner. Finally, at least 100 Leukocytes per smear were differentiated into a 20-category scheme by trained cytomorphology examiners, yielding over 18,000 individual cell images. This dataset forms the basis for training and validation of a convolutional neural network in order to allow independent recognition of malignant and non-malignant cell populations relevant in AML diagnostics. A sufficient amount of high-resolution microscopic images and high-quality annotation data are key requirements for successful application of Deep Learning to cell classification. When these prerequisites are fulfilled, plenty of algorithmic techniques are at hand to improve automatic cell recognition for standardised, objective cell differentiation.

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Poster Title	The challenge of PD-L1 clinical testing in NSCLC: current standards, sampling considerations and a future opportunity for RNAScope and digital pathology?
Abstract	Checkpoint blockade therapy is a new paradigm in cancer treatment with durable tumour regression and prolonged stabilization of disease in patients with advanced cancers, including non-small cell lung cancer (NSCLC). We present here a comprehensive assessment of the PD-L1 IHC diagnostic test in NSCLC at different levels. 1) A comparative validation of two antibodies, 22C3 (Dako) and clone SP263 (Ventana). 2) A description of clinical PD-L1 testing as a reflex test in the first 564 cases in an accredited laboratory. 3) The concordance of PD-L1 overexpression by IHC versus PD-L1 upregulation by RNA ISH. 4) The role of digital pathology in the automated scoring of PD-L1 IHC using QuPath digital pathology software. 813 NSCLC tumour samples collected prospectively from 564 diagnostic samples and 249 retrospective samples were analysed. Validated methods for IHC and RNA-ISH were tested in TMAs and full sections and digital analysis conducted with QuPath. Antibody validation concordance of SP263 and 22C3 was 97-98% in squamous cell carcinoma and adenocarcinomas, respectively. Clinical NSCLC cases were reported as PD-L1 negative (48%), 1-49% (23%) and >50% (29%), with differences associated with tissue-type and EGFR status. Comparison of IHC and RNA-ISH was highly concordant. Comparison of digital assessment versus manual assessment was highly concordant. Discrepancies were mostly around the 1% clinical threshold. Challenging IHC interpretation included a) calculating the total tumour cell denominator and the nature of PD-L1 expressing cell aggregates in cytology samples; b) peritumoral expression of positive immune cells; c) calculation of positive tumour percentages around clinical thresholds; d) relevance of the 100 malignant cell rule. Sample type and EGFR status dictate differences in the expected percentage of PD-L1 expression. Phenotypic analysis of PD-L1 can be challenging, and interpretative guidelines are here provided. PD-L1 evaluation by RNA-ISH and digital pathology appear to be reliable, particularly in the adenocarcinoma subgroup.

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