BOOK OF ABSTRACTS

13TH EUROPEAN CONGRESS ON DIGITAL PATHOLOGY

– HOSTED BY GERMAN SOCIETY OF PATHOLOGY –

MAY 25TH–28TH, 2016

LANGENBECK-VIRCHOW-HAUS

BERLIN, GERMANY

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SP01.05 | **Opening**

HISTORY OF THE EUROPEAN CONFERENCE SERIES ON DIGITAL PATHOLOGY: MEMORIES AND PERSPECTIVES

K. Kayser*
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Introduction/ Background
The 13th European Congress on Digital Pathology is the most recent conference of a European Conference series that started in Heidelberg 26 years ago. It reflects to a continuous, unbroken exchange of technological and medical information. The digital world was still in its childhood at the date of the first conference. Technological research investigated in electronic communication and digital acquisition of coloured pictures. Frozen section services and its need for fast information transfer between different institutes and the surgical theatre dominated the application of technological development. Consecutively, all issues of telepathology were in focus at the start and the following conferences. The pioneers of that time tried to convince their colleagues of the promising perspectives and the increasing technological influence on pathology.

It took several conferences in this series until the majority of or nearly all pathologists recognized the power of this new technology. Retrospectively, some conferences remained at the scientific level of their preceding meetings, whereas others substantially promoted knowledge and application in research and routine pathology.

At present, digital pathology is well implemented and mainly used for education and enhancement of molecular biology methods such as next generation sequencing, predictive diagnosis, or risk associated investigations. Implementation in routine diagnostic pathology (virtual slides, etc.) is on its way.

In addition, digital pathology moves forward to explore still unknown areas in surgical pathology, and tissue – based diagnosis. These include considerations on morphology, function and order of structures, which can detect potentially endangered factors or repair of live threatening breakdowns, as well as biostatistics, data mining, or self recognition algorithms.

KN01.01 | **Keynote I — Horst Karl Hahn**

COMPUTER ASSISTED MEDICAL IMAGING: FROM RESEARCH TO CLINICAL APPLICATION

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Evolution or revolution? Medical imaging is in a transition from a qualitative to a fully digital, quantitative discipline with fast growing numbers of images and increasing need for multimodal, multidisciplinary assessment. Computer assistance is a key ingredient to efficiency, safety, and quality of care in this domain. With the availability of prior scans in digital format, and with the diversification of the clinical application domains, both data complexity and requests for specialized and integrated tools are rapidly increasing.

We will compare radiological imaging with the situation we currently face in digital pathology and discuss major implications and challenges for the future development of computer assisted medical imaging as key technology for modern healthcare. We cover both methodological research and clinical adaptation, ranging from modular and prototypical software integration to prospective clinical evaluation and medical device product integration. For any clinical use of new solutions, quality assurance is performed according to the guidelines defined by the FDA and other regulatory bodies, which represents a particular challenge for novel data driven and self-learning software systems. In addition, decentralized infrastructures including cloud computing will support efficient collaborative research.

Medical image computing, quantitative analysis, automated detection of clinically relevant findings, and computerized classification of disease patterns is expected to have major impact on solutions and workflows used in future clinical routine and in large clinical trials. For digital pathology, the automated and robust combination of information across multiple slides will be a key success factor. A major challenge rests on the barriers and costs of validation of a variety of new solutions and their integration into the routine clinical workflow.

IMPACT OF TISSUE SAMPLING ON ACCURACY OF KI67 IMMUNOHISTOCHEMISTRY EVALUATION IN BREAST CANCER

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Introduction/ Background

Gene expression studies have identified molecular subtypes of breast cancer with implications to chemotherapy recommendations. For distinction of these types a combination of hormone receptors and proliferative activity of tumor cells, estimated by Ki67 labeling index from immunohistochemistry (IHC) is used. Clinical studies are frequently based on IHC performed on tissue microarrays (TMA) with variable tissue sampling. This raises the need for evidence-based sampling criteria for individual studies. We present a novel tissue sampling simulation model and demonstrate its application on Ki67 assessment in breast cancer tissue taking into account its intratumoral heterogeneity.

Aims

The aim is, using a novel method for easy virtual TMA simulation, to determine the optimal tissue sampling requirements in the context of variable intratissue heterogeneity level of Ki67 immunohistochemistry in breast cancer.

Methods

Whole slide images (WSI) of 297 primary breast tumors immunohistochemically stained for Ki67 were subjected to digital image analysis (DIA). Percentage of invasive tumor cells stained for Ki67 were computed for hexagonal tiles super-imposed on the WSI. From this, intratumoral Ki67 heterogeneity indicators (Haralick's entropy values) were extracted and used to dichotomize the tumors into homogeneous and heterogeneous populations. Simulations with random selection of hexagons, equivalent to 0.75 mm circular diameter, were performed. The tissue sampling requirements were investigated in relation to tumor heterogeneity using linear regression and extended error analysis.

Results

The sampling requirements were dependent on the heterogeneity of the biomarker expression. To achieve a coefficient error of 10%, 5-6 cores were needed for homogeneous cases, while 11-12 cores – for heterogeneous cases. In mixed tumor population, 8 TMA cores were required. Similarly, to achieve the same accuracy, approximately 4,000 nuclei must be counted when the intratumor heterogeneity is mixed/unknown. Tumors at the lower scale of proliferative activity would require larger sampling (10-12 TMA cores, or 5,000 nuclei) to achieve the same error measurement results as for highly proliferative tumors. Our data show that optimal tissue sampling for IHC biomarker evaluation is dependent on the heterogeneity of the tissue under study and needs to be determined on a per-use basis. We propose a method that can be applied to determine the TMA sampling strategy for specific biomarkers, tissues and study targets. In addition, our findings highlight the importance of high-capacity computer-based IHC measurement techniques to improve accuracy of the testing.

MORPHOMETRIC ANALYSIS IN BENIGN, ATYPICAL AND INVASIVE BREAST LESIONS AND ITS CORRELATION WITH HISTOLOGICAL DIAGNOSIS, TUMOUR GRADING AND HER 2 OVEREXPRESSION

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Introduction/ Background
Computer image analysis has become an important tool in the pathology laboratory for quantitative morphometric analysis, which has several advantages over conventional visual assessment: Objectivity, reproducibility and the ability to detect changes not immediately apparent to naked eyes.

The nuclear and cytoplasmic alterations during the development of various breast lesions are the cornerstone for typing and grading of these lesions, including carcinomas. The current study was aimed at analyzing the morphometric parameters like mean nuclear area, mean cytoplasmic area, nuclear:cytoplasmic ratio in various breast lesions including benign, atypical and malignant cases, with and without lymph node metastasis.

Aims
The purpose of this study was to compare the morphometric parameters of various breast lesions on formalin fixed paraffin embedded (FFPE) tissue sections with conventional histological diagnosis, tumour grading and HER2 overexpression.

Methods
A total of 100 cases were selectively obtained from archival paraffin blocks. 75 cases were of breast tissues from wide local excision/mastectomy or excision biopsy specimens along with 25 cases of positive lymph nodes with metastatic deposits from infiltrating ductal carcinoma. FFPE sections were stained with conventional methods of haematoxylin and eosin (H&E) and examined for histological diagnosis. The sections with representative breast lesions were stained immunohistochemically to determine HER2 status. Morphometric analysis was performed on H&E sections.

Results
The current study showed that 'p' value was highly significant ($p<0.001$) for nuclear area and N:C ratio among different categories. The cytoplasmic area was found significant ($p<0.001$) in discrimination of benign from malignant lesions but not atypical from malignant lesions, indicating that cytoplasmic area alone is not a very reliable parameter.

Conclusion: The morphometric evaluation of breast lesions has proved to be a useful technique in tumour pathology and a valuable adjunct to histomorphology in rapid and accurate diagnosis of different breast lesions. As nuclear changes precede morphological changes, nuclear morphometry can prove beneficial in diagnosing the malignant changes, both at the earliest and with accuracy.

In our study we found that mean nuclear diameter and N:C ratio were higher in grade III tumors than grade I and grade II tumours. The difference was statistically significant.

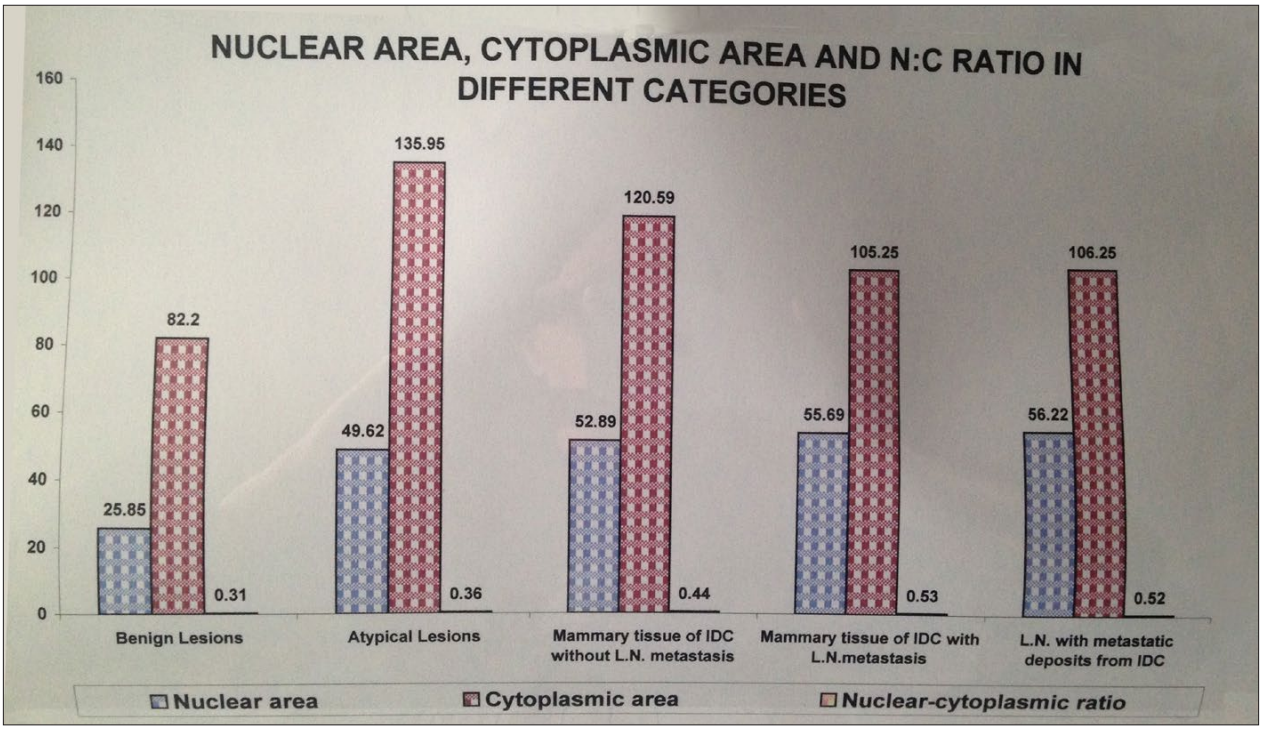
The malignant lesions with strong HER2 expression (3+) had higher mean nuclear area as compare to HER2 negative/ borderline(2+) tumours.

Thus, the correlation between histological diagnosis, tumour grading and morphometric nuclear parameters, in combination with HER2 overexpression, can improve the evaluation of the patient's prognosis, and possibly predict response to therapy.
Computer assisted nuclear morphometry can also be used in objective grading and standardizing grading performance between different laboratories. Hence nuclear morphometrical analysis will brings a factor of objectivity and help in quantification of the nuclear atypia and limiting the subjective variability in grading of breast cancers.

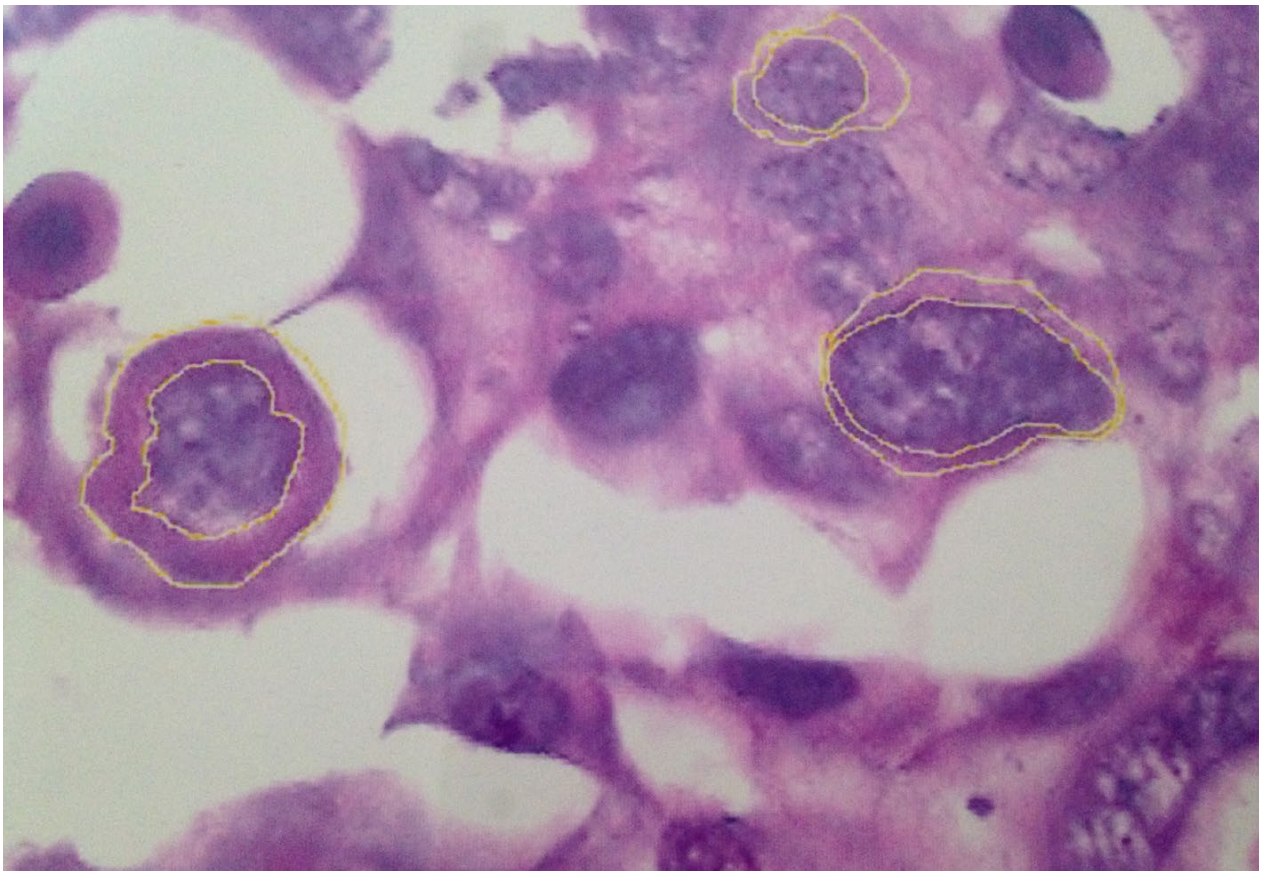
Tables, figures and microphotographs:

Category	No. of cases	Morphometric parameters			HER2 expression		
		NA(µm2) Mean±S.D	CA(µm2) Mean±S.D	N:C ratio Mean±S.D	Negative (Score -0, 1)	Borderline (Score -2)	Positive (Score -3)
Benign lesions	15	25.85±3.62	82.20±11.51	0.31±0.04	15	0	0
Atypical lesions	10	49.62±9.30	135.95±19.48	0.36±0.03	9	1	0
Breast tissue of infiltrating ductal carcinoma without lymph node metastasis	25	52.89±9.65	120.59±22.00	0.44±0.06	17	5	3
Breast tissue of infiltrating ductal carcinoma with lymph node metastasis	25	55.69±8.78	105.25±16.59	0.53±0.09	16	7	2
Lymph node with metastatic deposits from infiltrating ductal carcinoma	25	56.22±9.66	106.25±18.25	0.52±0.07	19	5	1

Correlation between histological diagnosis, morphometric evaluation and HER2 expression



Comparision of nuclear area, cytoplasmic area and nuclear:cytoplasmic ratio in different categories



Microphotograph showing morphometric measurements (nuclear and cytoplasmic area) in grade III infiltrating ductal carcinoma

SIGNIFICANCE OF MORPHOMETRIC ANALYSIS OF HPV-INDUCED CERVICAL DYSPLASIA

B. Vukomanovic Djurdjevic*
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Introduction/ Background
Genomic integration of high-risk human papilloma virus in the nucleus of cervical epithelial mucosal cells leads to epithelial dysplasia.

Aims
he aim of this study was to to establish the significance of morphometric analysis of the nuclear area in the assessment of the degree of cervical dysplasia

Methods
This study included 85 women with primary, previously untreated lesions, and colposcopic findings indicating dysplasia, in whom a cytological test by Papanicolaou method was interpreted according to the Bethesda criteria as low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and atypical squamous cells of undetermined significance (ASCUS). We performed human papilloma virus (HPV) typing by PCR for evidence of viruse types 16, 18, 31, 33. After biopsy of the cervical mucosa, we performed hematoxylin-eosin (H-E) and morphometric analysis of tissue samples. Morphometric analysis was performed on H&E stained slides at 400 x magnification. We analyzed four representative fields of dysplasia; 70 nuclei were photographed, using a digital optical microscope (Nikon Coolscope, Japan) and the morphometric computer program.The control group consisted of 10 women without dysplasia and without a verified infection of cervical high-risk HPV

Results
A high statistical correlation between the degree of dysplasia and the area of nuclei at different degrees of cervical dysplasias ($p = 0.000$). The high-grade cervical dysplasia had a more than 2-fold higher level of ranking in comparison to low-grade dysplasia, and a more than 10-fold higher ranking than the control group without cervical dysplasia. Our results suggest that the use the morphometric analysis are useful in the assessment of cervical epithelial dysplasias.

ANALYSIS OF COLOR STANDARDIZATION METHODS FOR THE AUTOMATIC QUANTIFICATION OF IHQ STAIN IN BREAST TMA

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Introduction/ Background

IHC biomarkers in breast TMA samples are used daily in pathology departments. This generates large amounts of information, which requires careful analysis [1]. Automatic methods to evaluate positive staining have been investigated since they may save time and reduce errors in the diagnosis that are due to subjective evaluation.

Aims

The aim of this work is to develop a density tool able to automatically quantify the positive brown IHC stain in breast TMA.

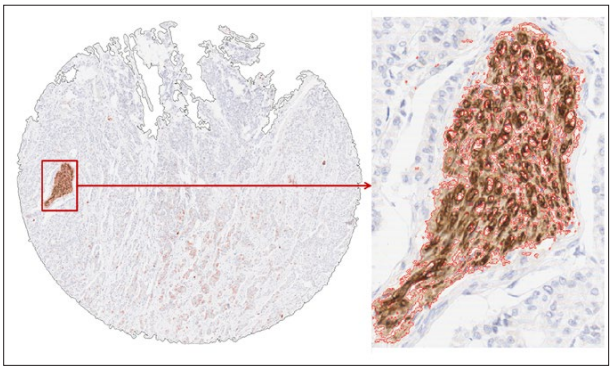
One of the problems when dealing with stained samples is color variation and distortion [2]. This is due to several factors such as the fixation process, the amount of stain or the digitalization process. One solution to the color variation problem is to apply standardization of reagents and procedures in histological practice. However, stains fade over time and therefore, it is not possible to achieve complete standardization with the current technology. In this paper, different methods for stain normalization have been analysed and compared in density quantification.

Methods

The methods implemented for stain normalization are based on the use of color distribution by means of dominant color descriptor, scalable and color structure descriptor. These algorithms adjust the color values of an image on a pixel-by-pixel basis so as to match the color distribution of the source image to that of a target image. Then, two main processes were performed to estimate TMA density: a) evaluation of total cylinder area and b) quantification of IHC stained area. For the 1st process, the algorithm distinguishes between normal, broken or distorted cylinders. The 2nd process deals with the evaluation of the positive brown pixels inside the cylinder. The segmentation is based on Lab thersholding together with binary thresholding applied to the H, S and B channels of the HSV and RGB color models. Finally, the tool segments all the positive areas and quantifies the brown density areas.

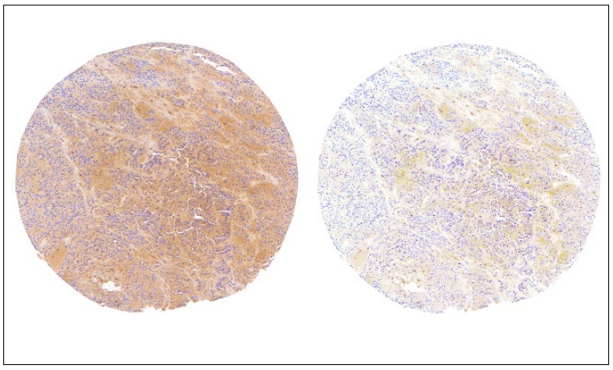
Results

A dataset of 879 TMA images were used to evaluate the methods. TMAs were prepared with an automatic tissue arrayer (Arraymold tool) composed of 50 holes/TMA with a cylinder diameter of 2mm. Slides were stained using different IHC stains, that is, CD1A, CD4, CD8, CD21, CD57, CD68, CD83, CD123, CK19, FOXP3, LAMp3 and S100. The acquisition of the digital TMA images was done with Aperio ScanScope XT scanner at 40x (0.25 µm/pixel). Afterwards, each cylinder image was individually extracted [3].



„Final result the positive IHC stain areas in red."

The use of color standardization makes the segmentation robust and free of parameter setting.



„Result of the colour image standarization."

Furthermore, the standarization process is able to reduce noise and facilitate the density quantification. The results were compared to manual density quantification by expert pathologists. The tests carried out provided up to 98% agreement when color standardization was applied againts 90% without color standardization. The biggest error comes from FOXP3 samples.

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[3] M. M. Fernandez-Carrobles, G. Bueno, Oscar Deniz, J. Salido, M. Garcia-Rojo, (2013), Automatic handling of tissue microarray cores in high-dimensional microscopy images, IEEE Journal of Biomedical and Health Informatics, 1–8

VALIDATION OF VIRTUAL DOUBLE STAINING FOR ESTIMATION OF KI67 PROLIFERATION INDICES IN BREAST CARCINOMAS

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Introduction/ Background
Ki67 is an important immunohistochemical marker of proliferation used in grading of breast cancer and endocrine neoplasms. Ki67 proliferation indices (PI) are calculated as number of Ki67 positive tumour cells divided by total number of tumour cells. However, manual counting and calculation of Ki67 proliferation index is laborious and prone to interobserver variability. Recently, a computerized algorithm that enables virtual alignment of two consecutive slides stained for pancytokeratin and Ki67 has been developed. Digital image analysis (DIA) based on Virtual Double Staining (VDS) enables exclusion of stromal cells and calculation of Ki67 PI in tumour cells only.

Aims
The purpose of this study was to validate the VDS algorithm by comparing manual counting and DIA on VDS for assessment of Ki67 PI in breast carcinomas.

Methods
Tissue Micro Arrays (TMA) were constructed with of 158 cores of breast carcinomas. Two slides were cut from each TMA and immunohistochemically stained for pancytokeratin and Ki67. For each core, between 2-20% of the total core area were selected for exact manual counting of Ki67-positive and negative cells using the stereological principle of systematic uniformly random sampling. A minimum of 200 cells were counted. The same areas were then counted using the VDS algorithm. Additionally, the VDS algorithm was used to calculate Ki67 PI for each core. In order two analyze the importance of the distance between neighbouring slides, five consecutive slides were stained for PCK. These slides were digitally fused and the percentage of overlap between stained and not stained areas calculated. Additionally, the VDS principle was used to examine differences in Ki67 PI when the immunohistochemical staining protocol was based on different antibody clones (Mib1, SP6 and 30-9) and staining platforms (Dako Autostainer, Leica Bond and Ventana Ultra).

Results
There was good correlation (R^2 >0.90, ICC >0.95) between manual counting in systematic randomized selected areas and DIA on VDS of both the whole core and in selected areas. Comparison of the two methods using Bland-Altman plots did not reveal any skewness in certain data ranges. Overlap agreement between neighbouring sections was on average above 88%, lower for diffusely infiltrating tumours than more solid tumours. Analysis revealed significant differences in calculated Ki67 PI between the different antibodies. The Mib1 and SP6 clones (concentrated format) were comparable on the Dako and Leica platform, while the average Ki67 PI for the SP6 clone was 44% higher on the Ventana platform. For the ready-to-use formats, Mib1 and MM1 based Ki67 PIs were 28% and 36% lower than average, while the clone 30.9 based Ki67 PI was 23% higher. In ***conclusion,*** DIA based on VDS may be an important future tool for improving accuracy and reproducibility of Ki67 PI in diagnostic and research settings.

DIGITAL IMAGE ANALYSIS OF HER2 IMMUNOSTAINED GASTRIC AND GASTROESOPHAGEAL JUNCTION ADENOCARCINOMAS

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Introduction/ Background

Manual assessment of HER2 protein expression in gastric and gastroesophageal junction (GGEJ) adenocarcinomas is prone to inter-observer variability and hampered by tumor heterogeneity and different scoring criteria. Cases are frequently reflexed to FISH.

Aims

This study aimed to evaluate the accuracy of digital image analysis (DIA) for the assessment of HER2 protein expression.

Methods

110 GGEJ adenocarcinomas were included in TMAs with 3 tissue cores per case. Two immunoassays, PATHWAY® and HercepTest™, and FISH were performed. The HER2 CONNECT™ DIA software as designed for breast carcinoma was applied. Connectivity, calculated by the software, was converted to standard IHC scores applying predetermined cut-off values for breast carcinoma as well as novel cut-off values.

Results

Applying HER2 CONNECT™ with established connectivity cut-off values designed for breast carcinoma resulted in 72.7% sensitivity and 100% specificity for the identification of HER2 positive cases. By application of new cut-off values, the sensitivity was increased to 100%, while the specificity remained 100%. With the new cut-off values, a 36-50% reduction of IHC equivocal cases requiring additional FISH analysis was observed.

Conclusion: HER2 CONNECT™ with adjusted cut-off values seems to be an effective tool for the assessment of HER2 protein expression in GGEJ adenocarcinomas, allowing for a decreased need for FISH analyses.

BRIDGE THE DISTANCE BETWEEN BREAST PATHOLOGISTS: WHEN THE SENOPATH NETWORK OPENS UP TO THE TELEPATHOLOGY

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Introduction/ Background

In clinical practice, pathologists commonly face breast lesions, which are difficult to diagnose and which require discussion. In Midi-Pyrénées, the largest region of France, this problem has led us to develop in 2011 a peer group for breast diseases entitled SENOPATH.

Aims

In order to reduce second opinion delay, erase geographical barrier and provide continuing education, we aimed to introduce an effective online and outline telepathology system in the SENOPATH network.

Methods

A case review by SENOPATH can be requested by any pathologist in the Midi-Pyrénées region, by filling a form through the ONCOMIP network (organization dedicated to oncology in the Midi- Pyrénées region). The slides are sent for digitalization at The University Cancer Institute - Oncopole, using a Hamamatsu 2.0-RS scanner (until 2014) and a 3DHISTECH Pannoramic 250 scanner, then anonymized and transferred to a shared storage space at Toulouse Paul Sabatier University. Virtual slides can be seen before and/or after the meeting by members of the group by login in the online 3DHISTECH Case Center via the Imag'IN platform website. The group, who meets on a monthly basis, has recently developed a synchronized webinar (using 3DHISTECH Case Center and Pannoramic Viewer) coupled with a conference call in order to ease the attendance of pathologists from remote pathology laboratories. A consensual diagnosis and final pathology report are issued for each case and sent to the referent clinician via the patient medical file securely hosted by ONCOMIP.

Results

From January 2012 to December 2015, 211 cases (39 in 2012, 50 in 2013, 75 in 2014 and 47 in 2015) have been reviewed during 43 meetings. Ten out of 43 meetings (23%) used telepathology. Sixty-one cases out of 211 (29%) were actually digitalized, mainly using the Hamamatsu 2.0-RS scanner. In average, the number of attending pathologists was 9 to 10 from 2012 to 2015. The average number of cases reviewed per meeting was 3 in 2012 and 5 between 2013 and 2015. Two main motives for review were identified: diagnostic 'routine difficulty' (equivocal or discordant cases, invasive vs in situ lesion, atypical vs malignant lesions, immunohistochemistry scoring pitfalls) or rare tumours. The rare tumour category included among others syringomatous tumour of the nipple, low-grade adenosquamous, myoepithelial, mucoepidermoid or secretory carcinomas, adenomyoepithelioma, atypical microglandular adenosis, sclerosing papillary hyperplasia without myoepithelium and periductal stromal tumour. Molecular analyses requested by the group and implemented in the diagnosis process mainly included immunohistochemistry and fluorescence in situ hybridization (HER2, ETV6, MAML2, MYB).

The SENOPATH network committee review for difficult or rare lesions of the breast has considerably improved the pathologists network in our region. This working group is regularly requested by oncologists to solve difficult cases. Our aims for the next few years are 1/ to digitalize all of the cases reviewed by the SENOPATH network, 2/ to use telepathology facilities provided by the Imag'IN platform in order to widen the group to a national level, and 3/ to construct a growing online library of virtual slides for breast challenging lesions.

COMPARISON OF DIGITAL AND CONVENTIONAL MEASUREMENTS OF THE MORPHOMETRIC PROGNOSTIC PARAMETERS IN CUTANEOUS MELANOMA

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Introduction/ Background

Measurements for Breslow and TNM staging on proliferations naevi and melanomas are required both by surgeons and patients, with direct interest in terms of prognosis and therapy. The advantages of digital measurements are: less time consuming, the ability to measure longer distances easy, as the possibility to extract meaningful images for clinicians, as they raise the question on the accuracy of the data supplied compared with those obtained in traditional transmission microscopy.

Aims

Cross comparison between conventional optical micrometer measurements versus whole scanned histological sections on paraffin tissue with malignant melanoma or naevi.

Methods

Digital measurements were performed on a series of cases of melanoma and nevi (n = 15) quantifying peripheral margins, deep margin, maximum tumor thickness, including the degree of invasion. Measurements were performed on standard HE staining sections, using Leica equipment (Aperioscan 2) and AperioImageScope 12.2 as software. Data were compared pursuing the gap between the two types of measures and the impact on TNM staging.

Results

The median numerical differences between the two measurements was low (between 0.003 and 0.023mm), the maximum registered was for depth of invasion. The variability was interpreted as human factor and training variability in taking measurements (most fluctuating - maximum invasion point). They have no significant impact in TNM staging scale Breslow and digital measurements allow a quantification of border areas, but with uncertain impact if we consider the tissue processing techniques induced changes. Digital measurements are advantageous because of its simplicity and speed, as well as calibration and standardization opportunities to reduce reading errors.

PROVINCIAL TELEPATHOLOGY NETWORKS AND THEIR CONNECTION INTO NATIONAL/CENTRAL TELEPATHOLOGY NETWORK IN CHINA: A RESULT ANALYSIS FROM 2012 TO 2015

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Introduction/ Background

In recent years, telepathology has played an increasingly important role in pathology consultation/second opinion. Since China implemented a national/central telepathology network for cancer diagnosis in 2011, many provinces have also planned and implemented their own provincial or local telepathology networks.

Aims

We here reported the provincial telepathology network and their operation from 2012 to 2015. We also reported their connection to national/central network.

Methods

All available provincial and local telepathology networks in China were surveyed. Only those provincial or local telepathology networks meet following criteria were included: (1) Network uses whole slide images and internet based platform technology; (2) Network sponsored or owned by provincial or local pathology quality control center; (3) Network currently functioning.

Results

From 2011 to Dec 2015, 6 provincial telepathology networks have been implemented. Xinjiang provincial network was set up in 2011 and connected to 40 local hospitals; Shandong provincial network(www.pathology.telemedicine.net.cn) was established in 2012 and connected to 50 local hospitals; Hainan provincial network (59.50.108.88) was set up in 2013 and connected to all 50 local hospitals in the province; Guangxi provincial network(www.gx.chinapathology.cn) was set up in 2013 and connected to 28 hospitals; Shenzhen city network (www.szcenter.cn) was set up in 2013 and connected to 15 local hospitals; Fujian provincial network (www.fj.mpathology.cn) was connected to 107 local hospitals. By 2015, 5 out of the 6 provincial networks have connected to national/central network. From 2012 to 2015, both national/central network and provincial networks provided pathology consultation for a total of 78,264 patients. Our survey indicated that in recent years multiple provincial telepathology networks have been set up in China. Through both national/central and provincial networks, telepathology consultation has played an important role in reducing diagnostic errors in China.

SY02.04 | **Telepathology**

TOWARDS EFFICIENT COLLABORATIVE DIGITAL PATHOLOGY: A PIONEER INITIATIVE OF THE FLEXMIM PROJECT

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Introduction/ Background

The development of digital resources for pathologists is a long process before truly validated algorithms can be used in daily practice.

Aims

In addition to developing new tools for helping Whole Slide Image (WSI) analysis by pathologists, the cooperative research project FlexMIm aims at setting up a shared platform allowing further technological improvements to be tested and evaluated online by a community of pathologists.

Methods

The FlexMIm consortium includes 27 pathology laboratories in the Paris area (coordinated by Assistance Publique-Hôpitaux de Paris), research laboratories from University Pierre et Marie Curie (UPMC Univ Paris 06) and University Paris Diderot, as well as 3 companies: TRIBVN, PERTIMM and Orange (project coordinator). Based on a cloud architecture, the project embeds a dedicated WSI database and visualisation support. Groups of partners developed dedicated algorithms. These algorithms have been tested separately, then integrated into the online platform. A large test and validation protocol, involving operational versions of these algorithms, is on-going among the 27 pathology laboratories participating to this project.

Results

One algorithm was built for blur detection in WSI in order to improve the quality of the workflow. Other quantitative algorithms were built for immunohistochemical counts such as Ki67, for mitosis detection from H&E (Hematoxylin – Eosin) stained WSI and for supporting the detection of Regions of Interest (ROI) for dysplasia screening in inflammatory bowel diseases (IBD). A series of algorithms (as gland detections in IBD) are at the proof of concept stage. Dedicated semantics and research engine are included in the platform, supporting the ROI collaborative annotations in WSI. The ontology used is generated by an operational contextual graph produced and validated for IBD diagnosis, consolidated by a semantic template linked to the annotations of IBD WSI. Another collaborative tool on the platform allows the online implementation of ontologies, with creation and edition of concepts. A full IBD diagnostic ontology is already available and a prostate cancer diagnostic ontology is underway. A major point of this platform is that all participating pathologists can finally evaluate online all the resources developed during the project. Anonymised WSI uploaded by each pathologist can be annotated by all other pathologists. These WSI can then be analysed by all the algorithms and tools available in the platform. Pathologists can eventually fill evaluation forms that are analysed by the project steering committee. Beside online resources, another goal of FlexMIm was to implement tools for faster WSI communication through networks especially in low bandwidth environments. The pathologists used a test bed in order to evaluate several compression algorithms on several visualisation devices (laptop, tablets), eventually leading to a "smart transportation" algorithm that can be activated in case of non-optimal network. Within 3 years, FlexMIm partners have thus built a platform which now integrates a whole set of algorithms to foster digital pathology adoption by a large cluster of Pathology laboratories.

SY02.05 | **Telepathology**

AN INNOVATIVE TELEPATHOLOGY SOLUTION FOR DEVELOPING COUNTRIES

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Introduction/ Background

The increasing incidence of pathologies like tumors and infections is a significant public health burden in developing countries. The ability to provide early diagnosis, treatments, follow-up care has a strong impact on the survival. Telemedicine is of great utility in countries lacking appropriate healthcare facilities by allowing for the performance of good level healthcare practices. Sub-Saharan African Countries suffer a dramatic shortage of medical pathologists (in the range of 1 to 10 pathologists per 10 million people) and are also victims of digital divide. Vittorio Tison Association (Tison), IRST research cancer hospital and Patologi Oltre Frontiera NGO (APOF) cooperate in the sanitary mission founded in 1999 in Bugando Medical Centre (BMC), a hospital located in Mwanza, Tanzania. In that mission, during 2011 we started the development of our telemedicine project. The project utilizes a novel telematic platform oriented to several sanitary branches with a special focus in pathology and oncology

Aims

The main project goals are:

- to provide ICT and TLC services between healthcare facilities in developed and developing countries
- to allow for simultaneous telepathology counselling sharing microscopy and radiology images, conference calling, remote diagnosis, double-blind evaluation, second opinion and the remote control of medical instrumentation
- to perform e-learning and remote quality control
- to carry out GCP clinical trials through data collection, monitoring and evaluation
- to encourage and support scientific research
- to reduce the knowledge gaps inherent to the digital divide

Methods

APOF has been developing the BMC pathology lab from 2000 to 2008. In the meantime Tison took care of training in Oncology of local medical doctors, opened the BMC Oncology Department in 2010 and patronized the building of a new clinic dedicated to the Oncologic Institute. We started the development of the project in 2011 with a general assessment of the needs and lacks in local working procedures, related to the possible improvements in ICT. Our first step involved the Internet connections activation and the implementation of the project informatic core in the IT room of the BMC Oncologic Institute. The telematic link between IRST's Italian site and BMC has been realized during the early pilot phase. We carried out several experimental sessions to investigate the compatibility of the main third-parts digital pathology products with our platform, choosing digital microscope Menarini D-SIGHT in association with D-SIGHT+ telepathology web-based application. Finally, on June 2015 we launched the BMC Telepathology Facility performing a complete demo of the system during the AORTIC East African Regional Meeting

Results

We validated the system in a wide range of conditions. Experimental data indicate an improvement of a factor up to 100 in the overall images transmission rate in comparison to the previous models. The pathology images remotely viewed are fully compliant with the diagnostic requirements in terms of definition and magnification. The platform is easy-to-use, all sanitary operators involved in the testing found it friendly and effective. The images browsing on the screen is very fast and precise, professional operators evaluated this solution equivalent to the use of the microscope. Our project is characterized by a high level of innovation which increases efficiency and efficacy of health practices and can boost the use of telepathology in developing countries.

MUCINOUS NEOPLASMS OF THE APPENDIX AND PERITONEUM : VIRTUAL MICROSCOPY FOR HISTOMORPHOLOGIC ASSESSMENT AND INTEROBSERVER DIAGNOSTIC REPRODUCIBILITY

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Introduction/ Background

Among gastrointestinal (GI) tumours, pseudomyxoma peritonei (PMP) from appendiceal origin has unique clinical and morphologic features. Due to the relative paucity of patients and the absence of therapeutic consensus, evaluation and refinement of the morphologic criteria used for assessment of the disease are still difficult. As a result, a uniformly accepted classification is still lacking. In collaboration with NJ Carr, who initiated the conference consensus process, in Basingstoke, and on behalf of the French group RENA-PATH, 11 experienced GI pathologists agreed to participate to a virtual workshop, in order to assess interobserver variability in PMP diagnosis and staging.

Aims

The goal of the study was to evaluate, for appendiceal and peritoneal mucinous neoplasms, the degree of concordance in the identification of diagnostic histological criteria by experienced pathologists, and to assess the degree of inter-individual variation in the application of WHO classification (2010) and TNM staging system (7th edition).

Methods

A single section stained with hematoxylin and eosin from 9 resected cases of mucinous neoplasms was selected by members of RENA-PATH. All digitalized at a maximum resolution (X40) using an HAMMAMATSU scanner system, to ensure that all participants evaluate exactly the same tumour areas; 1 to 16 questions were prepared for each case. On Teleslide web platform, interactive services provided by TRIBVN.

All submitted cases were then reviewed by a panel of 11 pathologists with specific expertise and interest in PMP. Data were analyzed using SAS program.

Results

Whole slide set evaluated by all participants; No abstention or "unknown diagnosis" for any submitted case.

Agreement for classification, WHO 2010

1. Appendiceal mucinous neoplasms: LAMN 83 %; mucinous adenocarcinoma 92%.
2. Peritonei mucinous carcinoma: Low grade 91.7%; High grade 91.7%.
3. Disagreement on the concept of High Grade AMN defined by low power architecture of LAMN + high grade cytology
4. Agreement for using pTNM classification (82%) in PMP

- Pushing Invasion (PI) and dissection by acellular mucin (DAM) in appendix wall, are not reproducible criteria, and need to be better defined.
- Criteria need to be redefined to use HAMN according to a majority of participants
- The identification of signet ring cells is not reproducible; the lesion needs to be better defined.
- Invasion of organs and pattern of invasion (broat-front invasion / classic by irregular glands or single cells with desmoplasia) are not reproducible criteria.
- Improvement in staging assessment is needed

Conclusion:

Although histopathological features of peritoneal disease are significant prognostic factors requiring pathologists to classify mucinous carcinoma peritonei (pseudomyxoma peritonei), reproducibility in interpretation must be improved. This international collaborative project allow pathologists worldwide to share their expertise and knowledge through a dedicated interactive workshop session. It is expected an improvement in the management of mucinous neoplasms of the appendix and peritoneum.

SY03.01 | **Data Integration and Modelling**

NEXT GENERATION BIOBANKING - FROM BIOREPOSITORY TO DATA INTEGRATION CENTER

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Introduction/ Background

Biobanks and their operators are gaining popularity. However, they face a range of growing challenges. Many of these biobanks used to be smaller and mid-sized collections of data and samples, but are now being developed into centralized networks within clinics and consortia. These large biobanks require the integration of as many sources of information as possible, including imaging data in addition to any relevant structured data. This is especially the case in the field of virtual microscopy. Data collection is increasingly being orchestrated by workflow based systems and must eventually be incorporated entirely into rule based IT-processes.

Aims

In order to achieve this goal, the Charité University Medicine Berlin, VMscope GmbH, and Kairos GmbH formed a consortium in the framework of the joint project, "Biobanking 3.0", which is funded by the German federal ministry for Education and Research (BMBF). Furthermore, after this successful project, a second KMU funded project called POST (Patient-centric open Multi Center Study Tool) will begin in the autumn of 2015 to expand the data integration to pathology information systems in a standardized manner.

Methods

One of the project goals is to use an IT-platform with an integrated workflow-engine to orchestrate all necessary processes and document all findings. Defined SOPs during sample extraction and information acquisition can be executed, and all result data can be made available to local research groups as well as project partners.

Results

Thus, the platform pursues the research approach of personalized medicine, which requires exact controlling of therapy cycles in a logical and chronological order.

SY03.02 | **Data Integration and Modelling**

BIOBANK SEMANTIC INFORMATION MANAGEMENT WITH THE HEALTH INTELLIGENCE PLATFORM

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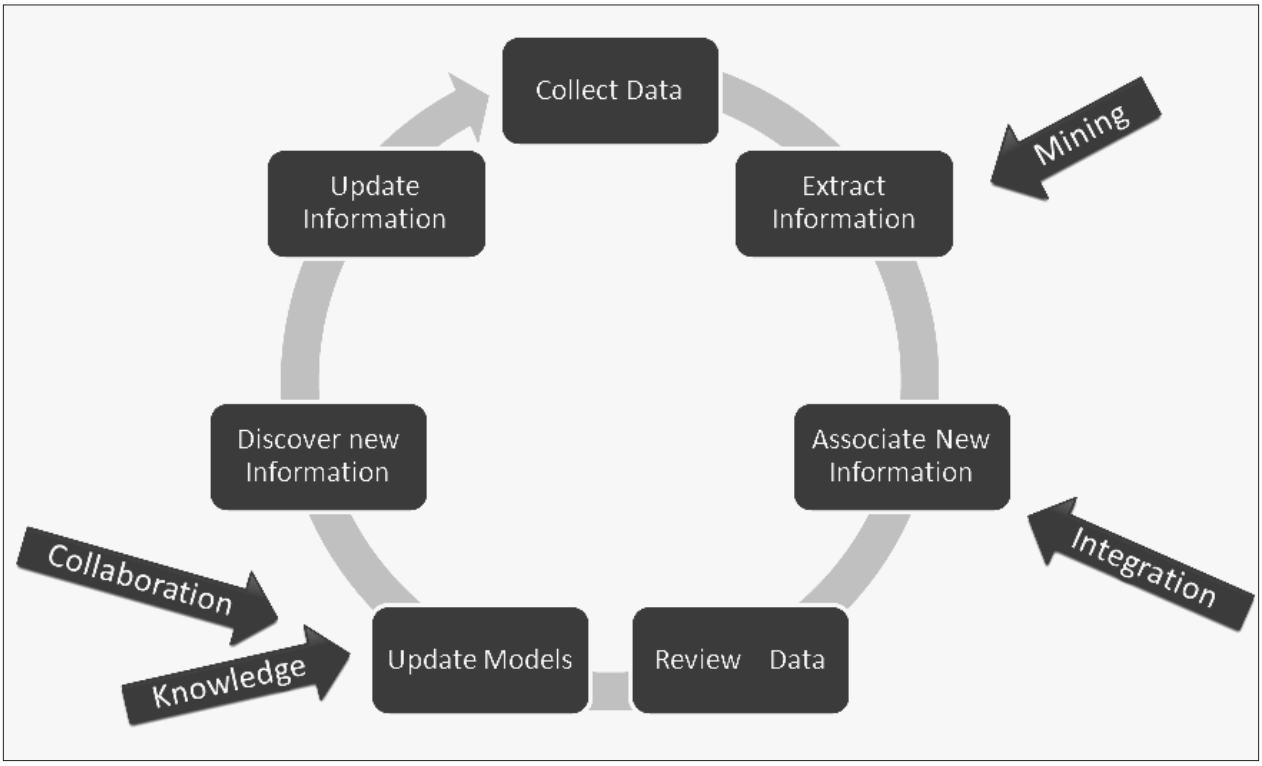
Introduction/ Background

Traditionally Biobanks were mostly used as repositories of cells and tissues. They are addressing scientific questions mainly for retrospective trials where stored tissues and cells can be used. This paradigm has been changing lately. Biobanks can play a different and more prominent role in the scientific process. In fact, the information contained in biobanks can fuel the scientific reasoning itself more than just accompanying it with management of specimens and data. Information management for biobanks should integrate all kinds of information from various sources including clinical records and documents to support flexible retrieval and analytics based on that information. One of the key concepts to support this is to treat the sample like the patient or donor. Selecting a sample for a research trial may be done based on real-time data from the clinical processes. This closes the loop and opens the iron curtain between clinical processes and clinical research. It is a key requirement for supporting research for precision medicine allowing for dynamic decisions and trial designs. Another important aspect is collaboration and sharing of important information preserving data security and patient safety. Informed consent should be dynamically integrated into the scientific process and support patient/donor literacy for the scientific question

Aims

More precisely we define requirements for biobank management systems to be

- Collaboration and global sharing of data
- Support for Scientific workflows and knowledge management
- Association of genotype and phenotype data and Integration of patient records
- Integration with registries and standards integration
- Support for informed consent



HIP Ontology Engineering with the Semantic Workbench

Methods

We present a biobank architecture based on our product 'Health Intelligence Platform (HIP)' that supports these requirements. We describe a way to support scientific reasoning directly with HIP and integrate sample management in a way that it contributes to scientific reasoning and outcome. The NLP semantic information extraction together with information extraction from (semi)structured sources provide a necessary integration to support the link between sample and clinical information. The semantic convergence model of HIP supports semantic queries based on an integrated semantic information base. Selecting samples or running analytics based on this information is possible in real time. The flexible knowledge management supports quick adaption of the knowledge base. Researchers are able to adapt the ontologies by visual interaction with the data sources and semantic tagging. The semantic workbench supports roundtrip engineering of ontologies and conflict resolution. We will present an evaluation framework that compares traditional biobank processes with HIP biobank management. We present the integration capabilities and provide an outlook to allow patient participation in research to address upcoming issues in precision medicine.

Conclusion:

Biobank management with HIP supports biobank management by providing tools and services for horizontal (across stakeholders, workflows and collaborations) and vertical (across institutions and disciplines) semantic integration of data into one common data model. HIP biobank management supports semantic retrieval of sample data and associated scientific and patient centered information. The HIP Core is used already and is used to identify patients for clinical trials based om information from clinical records and documents.

DATA ARCHITECTURE FOR A CLINCAL DATA REPOSITORY - EVALUATION AND DESIGN AT CHARITÉ UNIVERSUTÄTSMEDIZIN BERLIN

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Introduction/ Background

The translation of scientific results into new and more effective diagnostic and therapeutic procedures is a milestone in the advancement of medicine. German clinics collect large amounts of data on every patient which is used to: evaluate treatment; justify expense reports; operate quality assurance and to keep aftercare Physicians/caregiver informed. However, so far there are only fragmentary approaches to using this data resource. The combination of phenotype information from clinical routines with information about samples held in the biobank systems and linked with genetic information must be achievable. Therefore a data architecture must be designed which allows an access of all relevant patient data stored in different source systems under the aspects of data security, data protection, data integrity and sematic interoperability. The following paper focused on the integration of patient clinical data and patient sample data stored in the biobank system. The central role plays the design and implementation of a Clinical Data Repository (CDR) at the Charité.

Aims

Definition of the essential requirements of a Clinical Data Repository regarding data security, data protection, data integrity and sematic interoperability. Definition of standardized data flow from clinical patient system into the clinical data repository. Definition of the data model of the clinical data repository.

Methods

In order to design the central Clinical Data repository an investigation of existing clinical and research system landscape at the Charité took place. The existing solutions were evaluated regarding usage, level of penetration, standards in interoperability and supported interfaces. The analysis of Clinical Data Repository requirements based on the methodology of Requirements-Engineering. This methodology is very often used for development of complex IT systems in order to gain a common understanding on user side and developer side too. In 2013 and 2014 Charité supported a project to identify the main system demands on a clinical scientist workplace. The essential demands incorporated the Clinical Data Repository requirement analysis. From the technically point of view the Clinical Data Repository has to deal with a large amount of data in terms of storage, scalability, stability, fast accessibility and search and the support of data analytics. The introduction of In-Memory technology has enabled a paradigm shift in analytic applications, with many new possibilities. It is now possible to have all working data in the main memory, which means that internal database programming can be implemented to execute computer and data intensive algorithms without having to access data over slow interfaces. The Clinical Data Repository will be based on latest In-Memory technologies to allow researchers and physicians real time access to huge amounts of data.

Results

In the first phase the design of data architecture and a core set of clinical date is defined. A pilot implementation of a Clinical Data Repository will provide a research space where clinical data and research data is accessible for researchers and physicians. To correspond with data protection laws this data will be anonymized, pseudonymized and stored securely. The central biobank system is connected via an identify management system with the Clinical Data Repository. The CDR allows requests to identify groups of patient with include or exclude criteria from clinical workplace as well from biobank system.

CROSS-FERTILIZATION BETWEEN COMPUTATIONAL MORPHOGENESIS AND DIGITAL PATHOLOGY

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We believe that Computational Morphogenesis (CM) and Digital Pathology (DP) can be combined to provide valuable tools to study cancer, and improve patient diagnosis and prognosis. CM could play a research-oriented role in the quest to understand the biological and biophysical processes that underlie the different paths of cancer development. DP can play a key role for this task. In return, domain-specific validated CM Model (CMM) such as a stage I colon cancer model could assist routine DP.

Our aim is to give an outline of what DP can bring to CM and how DP can be enhanced by incorporating CMMs.

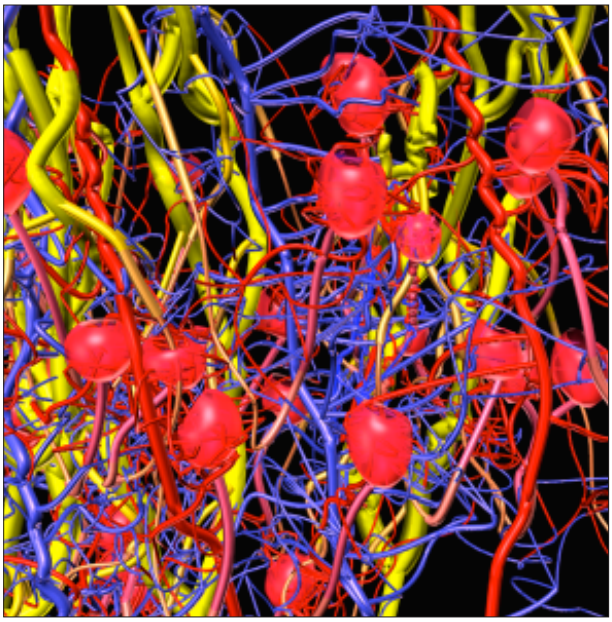
In order to have a CM platform that can be tailored to produce a specific CMM, the platform must allow specifying (i) cells lineages and their properties, (ii) their interactions via autocrine, paracrine and endocrine signals and (iii) their interactions with the extracellular matrix. We now describe how data from biology and DP can provide the necessary inputs to switch form a generic CM to specific CMM.

What DP can bring to CM

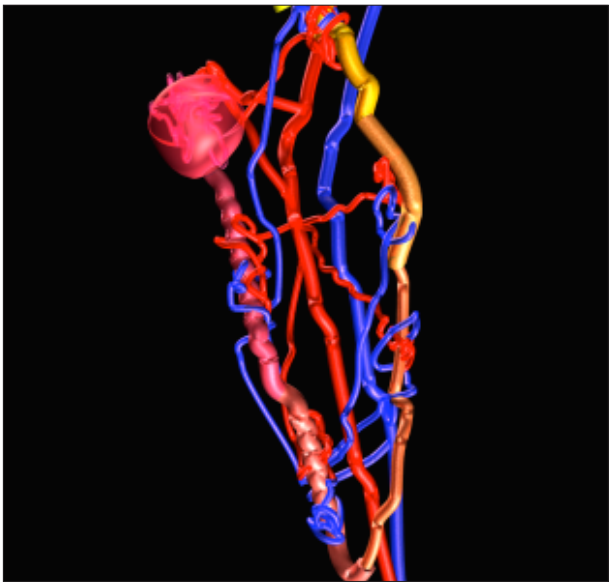
In cancer studies, cell lineages and their spatial organization can be obtained via tumor-associated (TA) biomarkers such as PD-L1 - PD1 [1] and CD86 - CTLA-4 [2]. Other biomarkers of particular interest include those that characterize TA and non-TA mesenchymal stem cells [3][4], fibroblasts [5], macrophages [6][7], and T cells [8]. Using 3D z-staked representations, the instantaneous snapshot of the spatial organization of specific cell lineages can instruct the modeling task, and different dynamic models can be built since TA and non-TA cells of a same general class (e.g. macrophages) can have distinct phenotypes such as promoting or inhibiting tumor progression [6][7]. Once the 3D spatial organization of the different cell-types is encoded into a CMM, reverse engineering and simulation can help determine putative initial tissue organization that may have led to the current tumor. Similarly, possible future states of the tumor can be predicted and then validated by comparing the simulated 2D/3D features by those obtained from real data. Hence, DP data could constitute one of the cornerstones of the iterative process of CMM specification, calibration, selection and validation.

Enhancing DP systems by integrating CMMs Validated CMM could enhance current DP systems for patient diagnosis and prognosis. Machine learning (ML) technics have been applied to analyze 2D WSI [8][9] and it is very likely that extending such methods to 3D tumor representations will improve the classification task. A large number of CMM-derived 3D reference models (3DRM) of, say, stage 1 colon cancer, could be produced as a complement to 3D models obtained by z-staking. The output of ML technics applied to real and simulated data could then be benchmarked by pathologists in order to assess if, when and how 3DRM can be integrated into decision-support systems dedicated to DP.

We have designed a prototype generic CM tool that has been tested to generate multi-scale models of vascularized kidney structures from virtual stem cells. It can be linked to a DP platform for further developments.



"multi-nephrons, collecting ducts"



"single nephron generated from virtual stem cells"

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BLOCK-CENTRIC VISUALIZATION OF HISTOLOGICAL WHOLE SLIDE IMAGES WITH APPLICATION TO REVEALING GROWTH-PATTERNS OF EARLY COLORECTAL ADENOMAS AND ABERRANT CRYPT FOCI

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Introduction/ Background

Comfortable navigation through diagnostic images is a prospective challenge for the acceptance of virtual microscopy applications in routine pathology [1],[2]. Tracing different regions of interest through multiple sections on one or several slides is a typical task in diagnostic slide examination. This laborious and time-consuming co-localization is currently executed by pathologists. Retaining the relative positions of tissue structures while alternating between multiple slides is still not feasible in a satisfactory manner in conventional nor virtual microscopy.

Aims

To address this issue we present a more comfortable and intuitive method to read slides using computer-assisted navigation. Furthermore, we demonstrate the strengths of our method by applying it to large series of serial colorectal tissue sections, creating new kinds of visualizations of different adenomatous mucosal architectures in human tissue, while looking for human correlates of lesions recently described in mice [3].

Methods

Histological images contain multiple distortions from different sources in the laboratory and digitalization process. An interconnection model was created to describe distortions by several layers, providing a normalized tissue representation. Layers were associated with specific distortions with each layer serving as a new level of abstraction. The first layers enabled a coarse alignment of tissue sections. Further alignment is achieved by piecewise, multi-resolution, SIFT-based [4] correspondence extraction and refinement. Inside the convex hull of all fiducial points local affine transformations were applied whereas a global affine transformation was used on the outside. Animated stacks were generated for regions of interest using local rigid transformations to preserve exact morphological coherences. For subsequent creation of 3D models, the relevant histological objects within these images were annotated by a pathologists, partly using computer assisted segmentation based on active contours [5]. These annotations were used subsequently to create simplified 3D models by applying VTK [6].

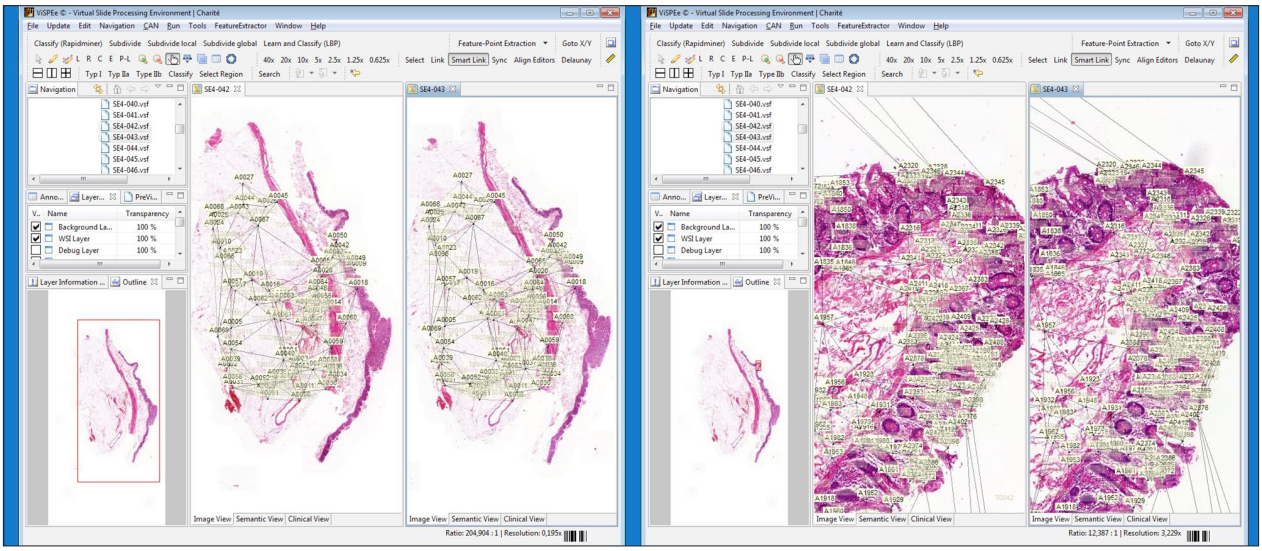

Figure 1: Feature-based, multi-resolution correspondence refinement

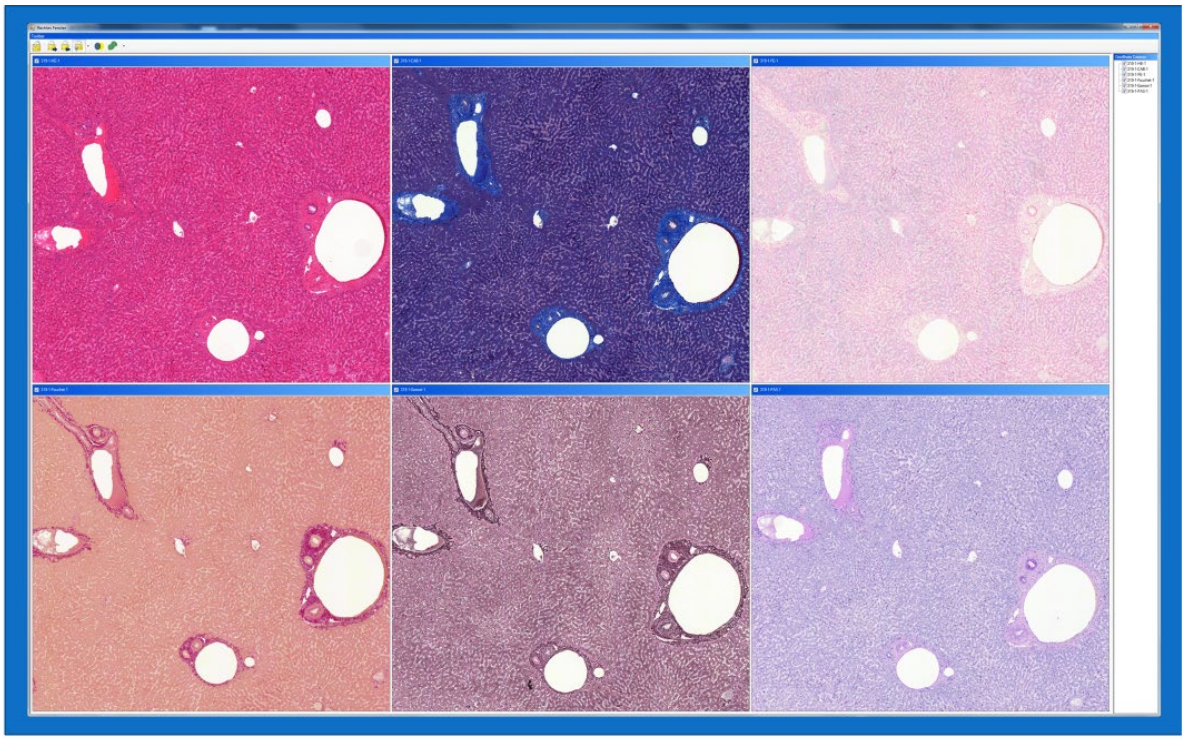

Figure 2a: Parallel, block-centric navigation through liver tissue (HE, CAB, FE, Fouchet, Gomori, PAS)

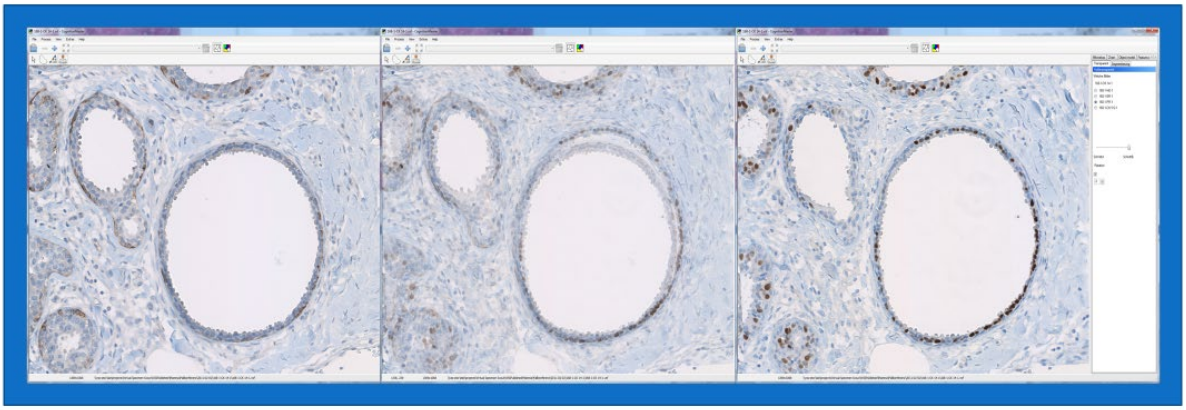

Figure 2b: Transparent overlapping of mammary tissue (CK14, CK14 and PR, PR)

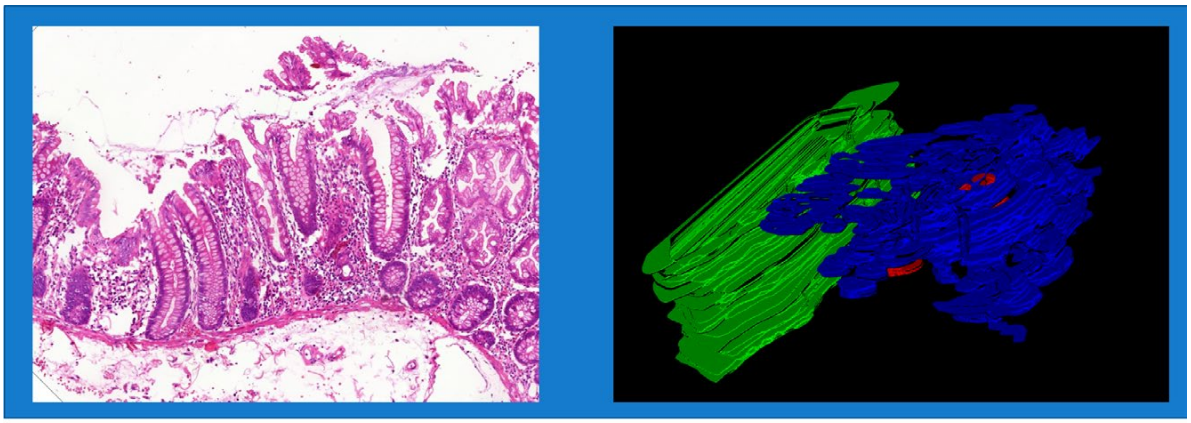

Figure 3: Advanced visualizations to reveal growth patterns of different adenomatous epithelia.

Results

The presented methods provide an efficient means to retrieve correspondences and additional spatial information from serial sections of histological slides. They also show good applicability for specimen from different origin. Alignment methods can be applied to generate block-centric visualizations such as parallel and transparent viewing of multiple stains. Moreover, the generated stack videos and 3D models demonstrate the very good accuracy of section alignment even in large series. The visualizations enable pathologists and researchers to grasp the 3D structural relationships in the tissue at a glance, providing an excellent tool to communicate more complex histomorphological findings. Interestingly, we see two kinds of tubular adenomas, which could imply multiple ways to tubular adenoma formation in FAP-patients, possibly akin to the recent observations in mice [3].

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INPUT DEVICE RESEARCH FOR DIGITAL PATHOLOGY. AN ERGONOMIC OUTLOOK

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Introduction/ Background

Digital Pathology represents a technological innovation that introduces changes in the traditional work of pathologists. In this regard, an important issue that has not been enough emphasized is the image handling from an ergonomic point of view to avoid work-related musculoskeletal disorders (MSD).

Aims

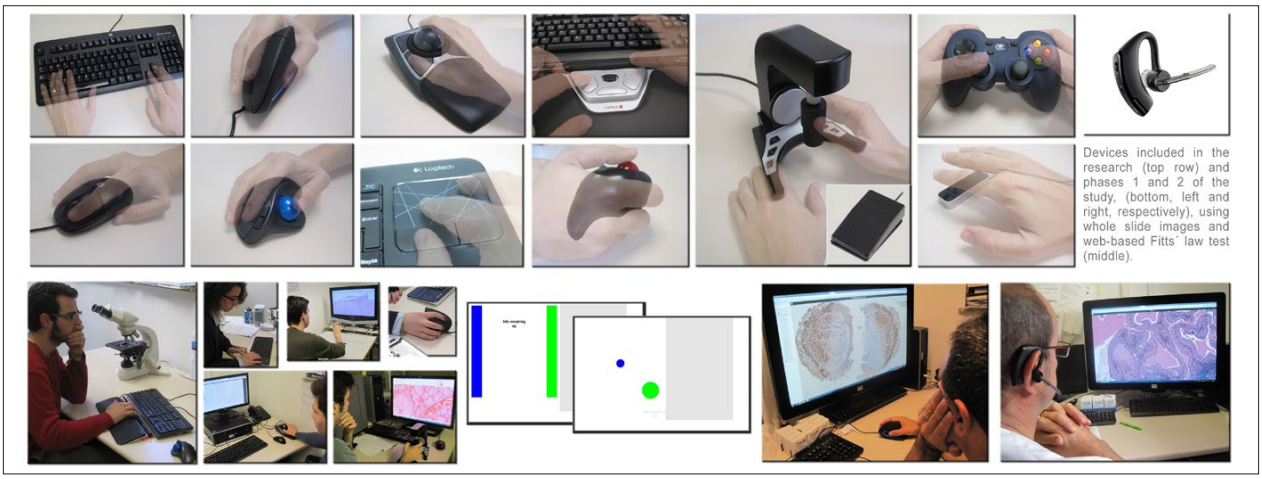
The aim of this study was to investigate a proper input device for digital pathology.

Methods

Research was conducted in two phases: 1. Comparative study to find an optimal external controller. Eight medical students analyzed 11 input devices: keyboard (HP), conventional mouse (HP), vertical mouse (CLS), touchpad (Logitech), 3 trackballs (Logitech, Kensington Expert and Ulove), Rollermouse (Contour), Ergopointer (Märzhäuser Sensotech), gamepad (Logitech) and a touchless device (Leap-Motion), using them with the Image Viewer software (Ventana). The web-based Fitts´ law test (UC Berkeley) was used to objectify the accuracy of each device, randomly. 12 items were included in the questionnaire: comfort, technical aspects (cursor movement and objective achievement), prospects, overall satisfaction, prior experience, and others. 2. Evaluation by two experienced pathologists (MPR and ANO, 55 and 50 year-old, respectively) the best rated input device and comparison with a voice recognition system (Invox Medical Dictation) using a headset microphone (Plantronics), rating perceived workload using NASA Task Load Index with 28 whole slide images. Digital Image Hub (Leica) with a 4 MegaPixel display (Barco) was used. Data were processed with SPSS 21.0.

Results

Correlation between technical aspects of the evaluated devices and accuracy (Fitts´ law test), and comfort with overall satisfaction existed ($p<0.05$). The assessment concluded that vertical mouse was the best rated input device. However, it has a slightly higher perceived workload in comparison with the voice recognition system, which was the proper controller for digital pathology in this study.



"Input devices and phases 1 and 2 of the research"

Conclusions:
We describe a methodology that can study and compare input devices for future workstations in digital pathology.

Pathologists should be involved in this process trying to find ergonomic devices that prevent MSD.

Voice recognition can function as a good handsfree device for digital pathology and could be considered in physical disability situations.

Further studies using electromyography, accelerometry and 3D reconstruction analysis could provide additional ergonomic information.

SY04.03 | **Virtual Microscopy**

PORTABLE VIEWERS FOR WHOLE SLIDE IMAGES

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Introduction/ Background
Whole slide images are the basis of actual Digital Pathology. We have not yet solved the problem of lack of standardization in image formats. Many different kinds of image formats coexist. Most of the time we use free viewers for local reviewing and complex servers for remote access. There is a tendency to integrate whole slide images inside hospital information environment with the rest of medical records. However this organization led to a loss of the physical management of the pathology images. We moved from the glass slides into a link in a server, and digital microscopy is really virtual microscopy.

Aims
With the aim of recovering the physical file archive for whole slide images to facilitate universal sharing of digital pathology images we evaluated the possibility of employing portable viewers from whole slide images. Technical difficulties have reduced the use of this type of viewers but they have the enormous advantage of the easiness of use and universal access without any installation, and display from any platform even from DVD. Open portable viewer are available and panoramic image photography generates a software technology that can be applied to Digital Pathology.

Methods
Four different source of whole slide images were used from four scanners: a Roche iScan Coreo, a 3D-histech Mirax Midi, a Leica SCN400 and a Dako ACIS III, getting .jp2, .mrxs, .scn and .tiff files. Aperio Image Scope viewer was used to read and convert files into three kinds of testing format tiff, jp2 and flat jpg files. A commercial Zeiss Java based software was used as portable viewer for tiff files. No direct jp2 portable viewer could be found available so flat jpg files were used instead to evaluate three other portable viewers: Zoomify, Deep Zoom and HD View. Zoomify has its own file converter from a single jpg into a image folder containing hundreds or thousands of small fragmented jpf files to reproduce the pyramidal file organization of whole slide images. Microsoft Deep Zoom Composer was used to generated the specific type of jpg fragmented pyramidal files and both hdmake command line software and Microsoft Image Composite Editor (ICE) was employ for HD View (HDV) files.

Results
All four tested solutions can be used as portable viewers and keep good image quality and easiness of use and distribution, independent of servers and software installation. However a previous work of conversion is necessary. The use of flat jpg files as source with its known file size limit of 65.000 pixels was overcame with ICE that works as stitcher software from different files. HDV works with zip files facilitating the use of a folder with a high number of small files. This solutions have for the future another standardization issue. They are using as visualizations technology Java, Flash and Silverlight. It seems that HTML5 will be soon substitutive standard, and probably has better security built but among its limitations is a bad solution as a local and portable active content viewer like whole slide images need.

CYTOMINE: AN OPEN-SOURCE SOFTWARE FOR COLLABORATIVE ANALYSIS OF WHOLE-SLIDE IMAGES

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Introduction/ Background
Major software for whole-slide image analysis and digital pathology are proprietary / closed-source which is not in line with current trends in open science and reproducible research. While some open-source software libraries exist for digital pathology (e.g. OpenSlide or NDPITools for reading and converting slide formats), to the best of our knowledge no open-source software exists that combine both remote visualization, collaborative and semantic annotation, and semi-automated analysis of digital slides.

Aims
Our Cytomine project started in 2010 to build a rich web environment for multi-gigapixel imaging data. This tool has been designed with the following objectives in mind: provide remote and collaborative principles, rely on data models that allow to easily organize and semantically annotate imaging datasets in a standardized way (using user-defined ontologies), efficiently support high-resolution multi-gigapixel images (incl. major scanner image formats), and provide mechanisms to readily proofread and share image quantifications produced by any image recognition algorithms. By emphasizing collaborative principles, our aim with Cytomine is to accelerate scientific progress and to significantly promote image data accessibility and reusability. We want to break common practices in this domain where imaging datasets, quantification results, and associated knowledge are still often stored and analyzed within the restricted circle of a specific laboratory.

Methods
Since the start of our project, we collaborated with biomedical researchers, pathologists, and computer scientists to shape the software and meet user and researcher needs. During our developments, we combined recent web, database, software development, and machine learning methodologies using open-source libraries. We also adopted modern practices (such as continuous integration and code quality testing) to build an industrial-grade software. In order to enable software extensibility and interoperability, we used a RESTful architecture so that e.g. other computer scientists can import/export data with their own algorithms and share their quantification results.

Results
The Cytomine software (http://www.cytomine.be/) has been released under an open-source licence since January 2016. In terms of code, Cytomine is composed of roughly 70K lines of code decomposed into its four main modules: Cytomine-Core (web server and database), Cytomine-WebUI (web user interface), Cytomine-IMS (image server), and Cytomine-DataMining (image recognition algorithms). Cytomine has now been used on various bio(medical) imaging datasets that involved various types of images and experts in different collaborative operating modes to perform various quantification tasks (in renal pathology, toxicology, developmental studies, lung and breast cancer research,...). By providing detailed documentation, installation instructions and source code, we hope that Cytomine will be used and extended for many purposes in digital pathology. Overall, we believe Cytomine is an important new tool of broad interest to foster active communication and distributed collaboration between pathologists, life scientists, computer scientists, teachers and students working with digital slides.

Related paper:
"Collaborative analysis of multi-gigapixel imaging data using Cytomine". Bioinformatics, DOI: 10.1093/bioinformatics/btw013, 2016.

A GLOBALLY OPTIMUM PARALLELIZABLE WHOLE SLIDE IMAGE REGISTRATION ALGORITHM

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Introduction/ Background

Advancements in imaging, communication and computing technologies lead to commercially available digital slide scanners for pathology. These devices help to increase the quality of consultation, diagnosis, research, education and archiving processes as well as contribute to cancer research. At this point, high resolution imaging of the whole pathological specimen is one of the most important parts of the digitalization process. The imaged tiles must be perfectly combined to create the While Slide Image (WSI). In the literature, there are publicly available registration schemes to create the WSI [1] [2]. These methods mainly suffer from the high computational times and high computational resource usage (mainly RAM). To overcome these weaknesses, we have developed a novel, fast, globally optimum WSI registration algorithm.

Aims

In this study, we plan to develop a new globally optimum WSI registration scheme for digital pathology. Time consumption, RAM usage, starting tile invariance and compliance to parallel processing are selected as the main design concerns of the registration algorithm.

Methods

The proposed registration scheme has 2 main steps: In the first step, Phase Correlation Method (PCM) given in [3] is utilized to compute the translational offsets between an image with its 4-neighbors. Since PCM requires computing Fourier and inverse Fourier transforms, only overlapping parts of neighbors plus an error region is used to reduce the amount of calculations. Then, all the translational offsets are stored in memory to be used in the global optimization step.

In the second step iterations are run to globally optimize the registration according to the neighboring tile relations. First, the priory knowledge from the motorized table is used to assign global coordinates to the tiles. Then, tiles best position is calculated by maximizing the translational offsets of the neighbors. Here, the tile coordinates are not updated, but the shift values both in X and Y coordinates are saved. After finishing the shift calculation process for all tiles, the shift map is updated, which concludes the first iteration. The iterations continue until zero shift is calculated for all images.

Results

The performance of the proposed scheme is evaluated using 2 basic scenarios. In the first scenario, 40 different pathology slides are registered using the proposed scheme and then stitched using a bilinear blender. The resulting WSI's are reviewed by 3 pathologists for stitching errors, and no faulty registration is recognized.

In the second scenario, registration time and maximum RAM usage are employed to evaluate the performance of the proposed scheme under different number of tiles. In the evaluations the proposed scheme is compared with Grid Stitching algorithm [1] implemented in ImageJ using the same tile sets and the same PC.

	Registration Time (min:sec) for 150 Tiles	Registration Time (min:sec) for 540 Tiles	Registration Time (min:sec) for 1890 Tiles	Max RAM Usage (MB) for 150 Tiles	Max RAM Usage (MB) for 540 Tiles	Max RAM Usage (MB) for 1890 Tiles
Grid Stitching (RAM Opt.)	01:01	03:36	14:37	1567	5133	15427
Grid Stitching (Speed Opt.)	00:39	02:06	08:26	1721	5380	17559
Proposed Scheme	00:28	01:41	06:57	217	849	3641

Comparative processing time and RAM usage performance of the proposed scheme

The results given in below table show that the proposed scheme has significant RAM and processing time advantages which are very important for real-world applications.

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SY05.01 | **Computer Aided Diagnosis**

STRUCTURE, FUNCTION, AND PREDICTIVE DIAGNOSIS ALGORITHMS

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Introduction/ Background

Background: Predictive diagnosis (PD) is a component of tissue based diagnosis. It is based upon immunohistochemical (IHC) and molecular genetic (MG) measurements of structures and functions. It predicts the outcome of individual cancer treatment.

Aims

To develop computerized analysis of microscopic images in relation to prognosis evaluation of cancer patients.

Methods

Theory: Structures are related to object – associated visual information that remains constant within the period of observation. Functions display with changes either in relation to contemporary structures or to the background or to both. Structures are usually visible in hierarchical spatial order, functions in both spatial and time oriented order. Functions usually alter or destroy one or several structures at a certain order which might cause the breakdown of the whole system. Especially, if higher order structures are involved. In microscopy the idea can be mapped on the diagnostic sequence that starts with conventional diagnosis (adenocarcinoma) followed by IH (receptor expression, EGFR), and ends with MG (k-ras). PD can be automatically derived from analysis of digitized images; and of potential (therapeutic) interactions between the different images (steps). The sequence results in:

1. Analysis of image quality, and evaluation of regions of interest (ROI).
2. Assessment of an automated conventional diagnosis.
3. Analysis of IH expression and quantification.
4. Analysis of intra-cellular pathways either by IH or fluorescent techniques.
5. Analysis of therapeutic interactions and evaluation of prognosis.

Results

Interpretation and Experiences: Image quality evaluation and standardization are mandatory to assure constant quality and reproducibility of the analysis results, such as ROI finding, colour intensity, diagnosis assessment, and IH quantification. The automated investigation of MG pathways and the final PD classification are not problematic. Each component contributes to potential interaction (drug regime), which all together add to evaluate the patient's prognosis. Perspectives: Graph theory defining nodes by structure and edges by function seems to be an adequate tool to construct an algorithm, which can be embedded in an open access data banks of individually tuned predictive diagnosis systems.

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SUSTAINABLE FORMAL REPRESENTATION OF BREAST CANCER GRADING HISTOPATHOLOGICAL KNOWLEDGE

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Introduction/ Background

Recently, histopathology has seen the introduction of several tools such as slide scanners and virtual slide technologies, creating the conditions for broader adoption of computer aided diagnosis based on whole slide images (WSI) to reduce observation variability between pathologists. This change brings up a number of new scientific challenges such as the sustainable management of the semantics associated to the grading process, image analysis and annotation in order to facilitate pre-filled report generation. The College of American Pathologists cancer checklists and protocols (CAP-CC&P) [1] are reference resources for complete Anatomic Pathology (AP) reporting of malignant tumors. Current terminology systems for AP structured reporting gather terms of very different granularity [2][3] and have not yet been compiled in a systematic approach. Semantic data models are formal representations of knowledge in a given domain that allow both human users and software applications to consistently and accurately interpret domain terminology [4][5].

Aims

Our objective is to i) analyze the histopathological knowledge for breast cancer grading available in the reference CAP CC&P and ii) to build a sustainable formal representation of this knowledge based on existing biomedical ontologies in NCBO Bioportal [6][7] and UMLS semantic types [8].

Methods

Our methodology was first experimented in the context of two cancer grading methods for invasive (Nottingham system) and ductal in situ breast carcinoma. A corpus consisting of 5 texts or "notes" was first selected by an AP expert from the two corresponding CAP CC&Ps. From each note the expert also extracted a list of key concepts to be used as a "gold standard". We used NCBO Annotator [9] for automatic analysis of the corpus. Annotator supports the biomedical community in tagging raw texts automatically with concepts from relevant biomedical ontology and terminology repositories. The methodology used consists in:

i) Automatic textual analysis and annotation of the corpus based on the 417 ontologies available on the NCBO platform. We selected a subset of ontologies based on the number of identified concepts and evaluated their relevancy with respect to the gold standard.

ii) Semantic modeling of the automatically extracted concepts into a sustainable formal representation based on their UMLS semantic types.

Results

We identified NCIT, SNOMED-CT, NCI CaDSR Values set, LOINC and PathLex as the ontologies providing the highest number of annotated concepts. *Table 1* shows as percentages the coverages of the concepts of each note by the annotations of the 5 reference ontologies. Percentages can add to more than 100 for a single note due to the possible overlap in ontologies coverages.

Table 2 uses the same format when only concepts from the gold standards are counted to quantify annotations

From the list of extracted concepts, we made a preliminary formal representation of the histopathological knowledge based on the UMLS semantic types of concepts. *Figure 1* shows the so proposed semantic modeling in the context of tubular differentiation.

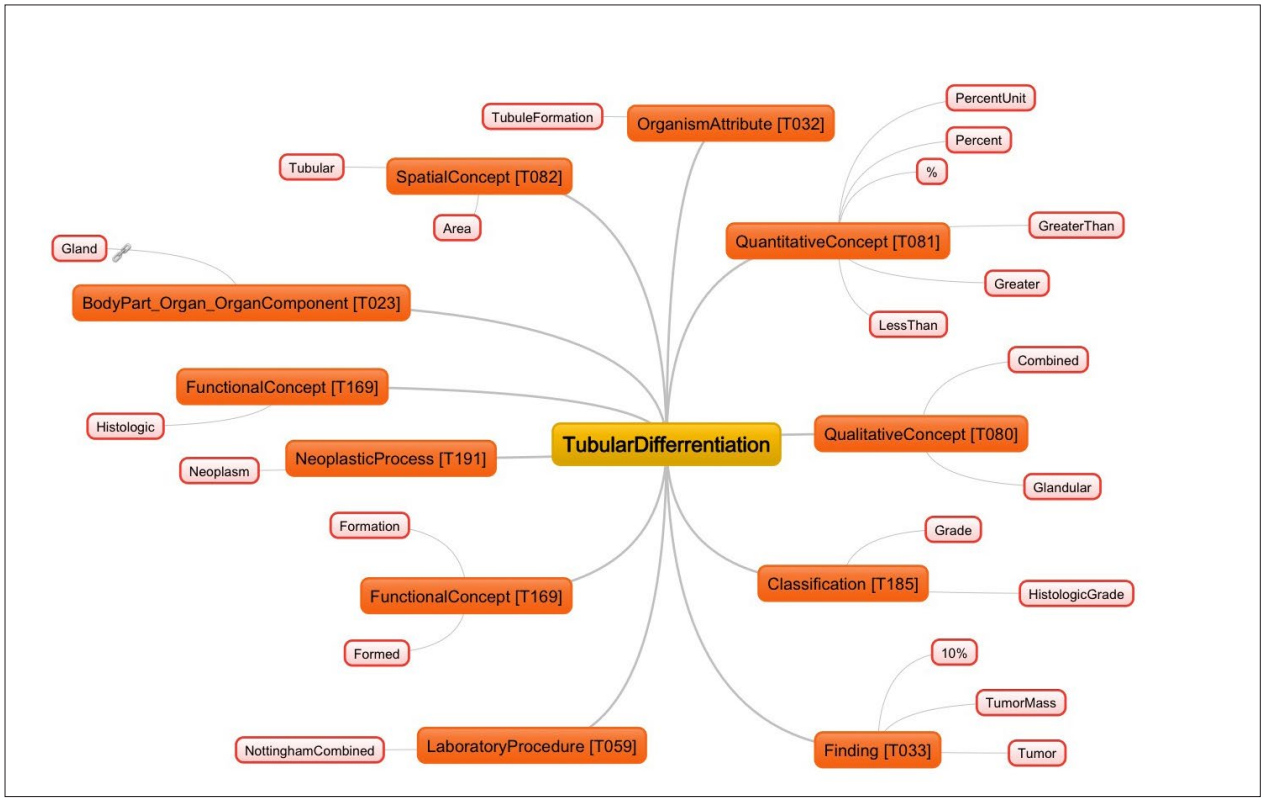
The novelty of this approach is the federation of the knowledge issued from different sources (CAP CC&P, NCBO ontologies and UMLS Metathesaurus) and the sustainable management of the associated semantics.

Gold standard		Ontologies									
		NCIT		LOINC		NCI caDSR Value Sets		SNOMED-CT		PathLex	
	Number of Concepts	Concepts	Coverage (%)	Concepts	Coverage (%)	Concepts	Coverage (%)	Concepts	Coverage (%)	Concepts	Coverage (%)
Note#1	**2**	**2**	100	**2**	100	**0**	0	**0**	0	**0**	0
Note#2	**8**	**4**	50	**0**	0	**0**	0	**0**	0	**0**	0
Note#3	**14**	**7**	50	**1**	7	**0**	0	**1**	7	**1**	7
Note#4	**5**	**4**	80	**3**	60	**1**	20	**1**	20	**1**	20
Note#5	**26**	**10**	38	**1**	4	**6**	23	**4**	15	**1**	4
	Total	**Total**	Average coverage (%)	**Total**	Average coverage (%)	**Total**	Average coverage (%)	**Total**	Average coverage (%)	**Total**	Average coverage (%)
	55	**27**	49	**7**	13	**7**	13	**6**	11	**3**	5

"Table 2: Number of concepts and coverages of the reference ontologies compared using the gold standard"

		Ontologies									
		NCIT		SNOMED-CT		NCI caDSR Value Sets		LOINC		PathLex	
	Number of Concepts	Concepts	Coverage (%)	Concepts	Coverage (%)	Concepts	Coverage (%)	Concepts	Coverage (%)	Concepts	Coverage (%)
Note#1	**23**	**15**	65	**10**	43	**5**	22	**6**	26	**1**	4
Note#2	**102**	**68**	67	**40**	39	**25**	25	**18**	18	**3**	3
Note#3	**58**	**31**	53	**28**	48	**15**	26	**12**	21	**6**	10
Note#4	**47**	**33**	70	**17**	36	**6**	13	**11**	23	**3**	6
Note#5	**103**	**71**	69	**31**	30	**29**	28	**12**	12	**3**	3
	Total	**Total**	Average coverage (%)	**Total**	Average coverage (%)	**Total**	Average coverage (%)	**Total**	Average coverage (%)	**Total**	Average coverage (%)
	333	**218**	65	**126**	38	**80**	24	**59**	18	**16**	5

"Table 1: Number of concepts and coverages of the reference ontologies in the annotation of observation notes of CAP CC&P"



"Figure 1: Tubular differentiation semantic modeling graphical representation"

This opens the perspective of building an AP observation ontology that will allow an accurate representation of AP reports understandable by both human and software applications.

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AUTOMATED IMAGE ANALYSIS OF HER2 FISH ENABLES NEW DEFINITIONS OF GENETIC HETEROGENEITY IN BREAST CANCER TISSUE

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Introduction/ Background

Therapy decisions for breast cancer rely on human epidermal growth factor receptor 2 gene (HER2) amplification testing. The HER2 status of tumors is primarily established by immunohistochemistry; borderline (2+) cases undergo further testing by fluorescence in situ hybridization (FISH). Current guidelines state that HER2 amplification is determined from manual counts of HER2 and CEP17 signals in 40 nuclei per case with an additional 20 nuclei in equivocal cases. The definitions are based on expert opinion rather than on objective statistical inference; they are complex, involving different quantities and cut-offs all at once (the signal counts and their ratio, proportion of cells amplified) and hard to follow. Automated image analysis (IA) extracts data from hundreds of nuclei and can aid HER2 testing in borderline cases.

Aims

We therefore explored if objective, statistically-derived indicators of HER2 heterogeneity can be obtained from automated HER2 FISH IA data.

Methods

50 cases of female invasive ductal breast carcinoma with HER2 2+ immunohistochemistry status, evaluated by the standard manual FISH methodology, were subjected to IA. The IA, developed using StrataQuest (TissueGnostics, Austria), segmented and counted individual nuclei and HER2 and CEP17 signals. A range of 192 to 789 nuclei per tumor were evaluated by the IA. All segmented nuclei and FISH signals were inspected manually for quality assurance and accuracy estimates. Bimodality indicator (Ashman's D) was computed for HER2 signal and HER2/CEP17 ratio in individual cells and included in factor analysis along with the data from manual and automated HER2 FISH analyses.

Results

No significant bias was found between the automated and manually corrected HER2 FISH nuclei or signals, obtained by IA. However, the manual HER2, CEP17 counts and HER2/CEP17 ratio were significantly underestimated by the automated procedure due to differences in cell selection in the techniques. By formal criteria (Ashman's D>2), 5 cases were classified as bimodal by HER2/CEP17 ratio and 23 cases by HER2 counts. Of those, 3 and 18 cases, respectively, were not amplified according to the cutoff of HER2/CEP17<2 by manual procedure. Factor analysis of the data set extracted 3 intrinsic factors of variation, representing amplification, "polysomy", and bimodality. Importantly, the factor scores could be seen as "purified" indicators, independent of well-known interactions between the absolute counts of HER2 and CEP17 signals per cell and their ratios. Therefore, the tumor cases may be independently characterized by the three vectors. Remarkably, the distribution of the tumors by the bimodality factor scores revealed a distinct peak of "highly bimodal" cases, suggestive of the possibility of robust stratification of the patients according to the bimodality indicators. We conclude that analysis of continuous HER2 FISH data obtained by IA enables new strategies for evidence-based stratification of heterogeneous breast tumors. In particular, indicators of bimodality of cell distribution according to their HER2 FISH signals may be useful in detection of heterogeneous cell populations, along with the currently used criteria based on cell proportions at a certain amplification cutoff. While clinical validity remains to be tested, we suggest that detection of bimodal distribution of cells can serve as robust, evidence-based stratification and decision support tool, highlighting potentially heterogeneous tumors.

LONG-TERM APPLICATION OF AUTOMATED KI67 QUANTIFICATION IN ROUTINE BREAST CANCER DIAGNOSTICS

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Introduction/ Background

The measurement of cell proliferation via Ki67 immunohistochemistry is an important part of tumor diagnostics leading to treatment decisions. The assessment according to the current guidelines is very time consuming which likely leads to counting only few cells or making the analysis completely estimated in many cases. Here, automated image analysis promises a standardized, time-saving and reproducible assessment.

Aims

We prior developed a computerized method that allows for an automated scoring of Ki67. This approach was validated with a study cohort of more than 1000 patients and showed a very high significance in both overall and disease free survival. The aim of this study was to test the capability of this method for the application in the daily routine diagnostics with the concomitant time pressure and huge variability in the patient material and staining results.

Methods

The developed computer algorithm and software was applied in daily routine breast cancer diagnostics under real conditions over more than one year. More than 100 cases have been analysed.

Results

The retrospective analysis showed that the method was capable of accurately processing most of the images. Problems occured when the the counter staining was to weak which made it even difficult to conventionally assess those images. A user interaction was required in some cases to exclude falsely counted non-tumor cells.

COMPUTER-ASSISTED INFLAMMATION ANALYSIS OF KIDNEY-GRAFT BIOPSY TO IMPROVE RISK STRATIFICATION IN ALLOGRAFT REJECTION

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Introduction/ Background

Kidney graft biopsy plays a key role in diagnosis of antibody-mediated rejection (AMR), the major cause of renal graft failure. The diagnosis of AMR requires the presence of i) donor specific antibodies (DSA), and ii) microvascular inflammatory lesions on kidney graft biopsy.

Aims

Histological assessment relies on Banff classification [1] that has quantitative and qualitative limitations and faces in terms of diagnostic accuracy and risk prediction: 1) this grading is categorial with risks of threshold effect; 2) the nature of inflammatory cells is not considered. Hence we propose a new method of computerized image analysis in order to finely characterize the quality and intensity of graft inflammation.

Methods

Data

57 kidney recipients fulfilled the Banff criteria for AMR between 2004 and 2012 at the Lyon Hospitals. Double immunohistological stainings were performed with CD31 (capillaries) and respectively CD68 (macrophages), CD3 (T lymphocytes), CD66b (granulocytes), CD20 (B lymphocytes). 288 glass slides were scanned (MiraxScan, 20x, NA=0.8).

Algorithms

The goal is to quantify the number of immune cells in the different parts of the kidney cortex. Due to the biopsy preparation such as biopsy slicing and image quality staining many variations within the observed object could happen and result to incorrect image segmentation. Hence we combine color component images to extract Regions of Interest (ROI) based on the use of their contextual data information in order to correctly extract the capillaries and immune cells. The algorithms are implemented in the Icy software (http://icy.bioimageanalysis.org) [2] . in the workflow: 1) color deconvolution [3]], 2) pre-processing step to segment the pixel staining 3) extraction of stained objects by combined information. The color deconvolution separates the initial image into 3 component-staining images: blue component for nuclei, the brown component for capillaries and the purple one for immune cells. These component images are first preprocessed, by gaussian filtering and then by the k-means classification to segment the images. We combine the segmented ROI and their spatial relation to extract the objects of interest.

Results

34 patients had C3d+ DSA and 23 had C3d- DSA. Although allograft survival was lower in the C3d+ group ($p<0,001$ by log-rank), Banff scores for AMR were similar in the 2 groups (3.4±1.1 vs 3.5±1.2, $p=0.65$). In contrast, our approach revealed notable differences in graft inflammation between the two groups. The number of CD68 cells in the capillaries and in the interstitium allows identifying patients with a risk of graft loss (HR=3.18, $p<0.01$ et HR=2.62, $p=0.01$ respectively). The combining C3d test and quantification of monocytes in interstitium allow clustering patients into 3 groups of renal prognosis: C3d-, C3d+/CD68 low and C3d+/CD68 high ($p<0.0001$ by log rank; C3d+/CD68 high vs C3d+/CD68 low: HR=2.43, $p=0.04$; C3d+/CD68 low vs C3d-: HR=4.99, $p=0.006$).

The isolated C3d test has an excellent value negative prediction (89,5% for the 1 year graft loss) but perfectible positive prediction (52,9%) [4] . The monocyte quantification allows to accurate the prognosis of patients in the C3d+ group. Using this novel reproducible approach for topological quantification of inflammation, we observed

that histopathological features of complement-binding DSA are different from that of non-complement. The computer-assisted analysis of graft inflammation improves the risk stratification of graft loss.

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A WORKFLOW FOR COMPUTER-AIDED CYTOLOGY IN WHOLE SLIDE IMAGES: APPLICATION IN FINE-NEEDLE ASPIRATION THYROID CYTOLOGY

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Introduction/ Background

Computer-aided cytology has a long history in computer vision research with a large amount of works in cervical cancer screening [Bengtsson et al. 2014 ; Delga et al. 2014]. However, one still lacks practical systems in many cytology fields due to several technical challenges (incl. the need for significant computational resources and the lack of efficient collaborative tools to efficiently collect and organize realistic and large ground-truth data that would enable large validation of recognition algorithms).

Aims

We present a novel workflow for computer-aided cytology using latest web, databases, and machine learning technologies with the aim to speedup the implementation of such systems. Here, we focus on specializing this framework and applying it for the assessment of thyroid nodules. In practice, physicians have to efficiently stratify patients according to their risk of malignancy in order to identify the best follow-up and therapeutic options. Fine-needle aspiration (FNA) and cytological assessment has become the predominant method used for the primary diagnosis of benign and malignant thyroid nodules, resulting in the categorization of patients as operative or non-operative candidates.

Methods

Data: FNAs were carried out using a 21-gauge needle attached to a 10-mL syringe. The aspirated material was smeared on slides, air-dried and subjected to a Diff-Quick stain. FNAs were scanned (Hamamatsu scanner, 40X, 0.23µm) at the ULB. Digital slides were transferred to a Cytomine (http://www.cytomine.be/) [Marée et al., 2016] server at the ULG.

Algorithms: Our framework is generic so that one can specialize each of its component. Here, color deconvolution [Ruifrok & Johnston 2001] is first applied on original image tiles to detect cells and separate them from background/artifacts. Detected foreground objects are then separated into individual cells or clusters. Localization of individual cells within clusters is then performed using Watershed and the distance transform. All individual cells are then classified using variants of our image classification algorithms. This binary model was trained and optimized using our ground-truth dataset of individual cells where we considered papillary cells with inclusion as positive and all other types of objects (cells with ground glass nuclei, nuclear grooves, normal follicular cells, artefacts, macrophages, polynuclear, ...) as negative. Similarly, large clusters are classified using a binary model optimized on the ground-truth dataset to discriminate between normal and proliferative follicular architectural patterns.

Results

Using Cytomine web annotation tools, experts first built an unprecedented ground-truth dataset of various types of normal and abnormal cells and clusters (> 6000 objects from 60 FNA whole-slide images) to train recognition models. Once all cells of new slides are classified by our workflow, predictions are uploaded to the Cytomine-Core server through HTTP requests and can be displayed in the Cytomine-WebUI as sorted galleries of most suspicious objects. At the conference we will present our qualitative and quantitative evaluation of the different steps of the workflow and discuss limitations and perspectives. This novel Cytomine module will be released as open-source in the near future so that other research groups will be able to train, apply, and extend it on their own data.

APPLICATION OF RAMAN MICROSCOPY FOR THE DIAGNOSIS OF THE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL).

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Introduction/ Background

In hematology, actual diagnosis of B chronic lymphocyte-leukemia (CLL) is based on the microscopic analysis of cell morphology from patient blood smear. However, new photonic technologies appear promising to facilitate and improve the early diagnosis, prognostic and monitoring of personalized therapy. The development of automated diagnostic approaches could assist clinicians in improving the efficiency and quality of health services, but also reduce medical costs.

Aims

The M3S project aims at improving the diagnosis and prognosis of the CLL pathology by developing a multimodal microscopy platform, including Raman spectrometry, dedicated to the automatic analysis of lymphocytes.

Methods

Blood smears were prepared on glass slides commonly used in pathology laboratories for microscopy. Two types of sample per patient were prepared: a conventional blood smear and a deposit of "pure" lymphocyte subtypes (i.e. normal B, CLL B, T and NK), sorted out in flow cytometry by using the negative double labelling technique. The second sample is used for the construction of a database of spectral markers specific of these different cell types. The preparations were analyzed with the multimodal machine which combines i) a Raman micro-spectrometer, equipped with a 532nm diode laser excitation source; ii) a microscope equipped with 40x and 150x lenses and a high precision xyz motorized stage for scanning the blood smear, and localizing x-y coordinates of representative series (~100 for each patient) of lymphocyte cells before registering three Raman spectra; these cells of interest being previously localized by an original method based on the morphology analysis. After the Raman acquisitions, the conventional blood smears were submitted to immuno-labelling using specific antibodies. For the establishment of the Raman classifiers, this post-acquisition treatment was used as reference to distinguish the different lymphocyte sub-populations. Raman data were then analyzed using chemometric processing and supervised statistical classifiers in order to construct a spectral library of markers highly specific of the lymphocyte type and status (normal or pathological).

Results

Currently, a total of 60 patients (CLL and healthy) were included in the study. Various classification methods such as LDA (Linear Discriminant Analysis), PLS-DA (Partial Least Square Discriminant Analysis), RF (Random Forest) and SVM (Support Vector Machine), were tested in the purpose to distinguish tumoral B lymphocytes from other cell types. These classification algorithms were combined with feature selection approaches. The best performances were around 70% of correct identification when a three-class model (B-CLL vs B-normal vs T and NK lymphocytes) was considered, and 80% in case of a two-class model (B-CLL vs B-normal lymphocytes). These encouraging results demonstrate the potential of Raman micro-spectroscopy coupled to supervised classification algorithms for leukemic cell classification. The approach can find interest more generally in the field of cyto-hematology. Further developments will concern the integration of additional modality such as Quantitative Phase Imaging on one hand to speed the exploration process of cells of interest to be probed, and on the other hand to extract additional characteristics likely to be informative for CLL diagnosis. In addition, the identification of prognostic markers will be investigated by confronting the photonic data to clinical patient information.

FEATURES OF AN EXPRESSION OF SMOOTH MUSCLE ACTIN BY MUSCLE CELLS OF ARTERIOLES AND VENULES OF URETER AND URINARY BLADDER OF NEWBORNS IN MODELING CHRONIC INTRAUTERINE HYPOXIA

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Introduction/ Background
Chronic intrauterine hypoxia, accompanied numerous complications of pregnancy, is one of the damaging factor of the organism of fetus and newborn.

Aims
The purpose – to reveal the features of expression of smooth muscle actin by muscle cells of arterioles and venules of ureter and urinary bladder of newborns under the influence of experimental chronic intrauterine hypoxia.

Methods
An experiment was conducted on modeling high mountain hypoxia in rats of WAG line. Two groups were formed: I – newborns (n=11) born from 3 rats that were not subjected to high mountain hypoxia; II – newborns (n=10) born from 4 rats that throughout pregnancy were exposed to a daily high mountain hypoxia. The material of the study was the tissue of ureter and urinary bladder. It was used peroxidase reaction with the monoclonal antibodies to smooth muscle actin (DAKO, Denmark). The slides were studied in microscope «Olympus BX-41». An experimental study was carried out in strict compliance with the requirements of the European convention (Strasbourg, 1986) on the housing, feeding and care of experimental animals, and their removal from the experiment. Mean values of indicators in groups were compared using a nonparametric U-criteria Mann-Whitney.

Results
In group I and II was observed expression of smooth muscle actin by muscle cells, forming muscle fibers and including in the tunica media of arterioles and venules of ureter and urinary bladder, in a clear cytoplasmic staining in brown. In group I the mean values of indicators of thickness of muscle fibers in arterioles and venules of ureter were respectively (2,19±0,229)×10-6m and (1,38±0,189)×10-6m; in urinary bladder – (2,92±0,207)×10-6m and (2,11±0,160)×10-6m. In group II the mean values of indicators of thickness of muscle fibers in arterioles and venules of ureter were respectively (1,67±0,270)×10-6m and (0,88±0,107)×10-6m; in urinary bladder – (2,20±0,198)×10-6m and (1,49±0,239)×10-6m. Analyzing and comparing the obtained values, it was observed that in group I and II was determined significant predominance ($p<0,05$) the mean values of indicators of thickness of muscle fibers in arterioles in comparison with venules in ureter and urinary bladder. In group I was a significant ($p< 0,05$) prevalence the mean values of indicators of thickness of muscle fibers in arterioles and venules of urinary bladder in comparison with ureter; significant differences in group II ($p >0,05$) were absent. In group II in ureter and urinary bladder was a significant ($p<0,05$) decreasing of the mean values of indicators of thickness of muscle fibers in arterioles and venules in comparison with group I. ***Conclusion:*** Chronic intrauterine hypoxia leads to a thinning of the thickness of the muscle fibers in arterioles and venules of ureter and urinary bladder in newborns.

APOPTOTIC ACTIVITY OF CELLS OF THYMUS AND SPLEEN IN CHILDREN BORN TO WOMEN WHO LED AN UNHEALTHY LIFESTYLE

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Introduction/ Background
The health of children largely depends on the health of parents and their lifestyle.

Aims
The purpose – to identify the apoptotic activity of cells of thymus and spleen in children born to women who led unhealthy lifestyle.

Methods
The material of the study was the tissue of thymus and spleen of children born to women who led a sedentary lifestyle, smoked, drank alcohol and ate foods containing tartrazine. Investigated material was divided into three groups: I – cases of stillbirth (n=14); II – cases of autopsy of children who died before the age of 6 months (n=38); III – cases of autopsy of children who died at the age from 6 months to 1 year of life (n=15). The cells in apoptotic state were detected using monoclonal antibodies to CD 95 in the fluorescent microscope «Axioscop-40». Immunohistochemical investigation was performed using the indirect Koons method in modification of M. Brosman (1979). Mean values of indicators in groups were compared using a nonparametric U-criteria Mann-Whitney.

Results
In thymus of children was observed a significant (p=0,000345) increasing the number of cells in apoptotic state in group II (35,61±0,701) in comparison with the group I (31,29±0,794) and in group III (42,13±1,073) in comparison with the group II (p=0,000014). In spleen was found a significant (p=0,010395) increasing the number of cells expressing receptor for CD 95 in group II (19,89±1,055) in comparison with group I (14,50±1,550) and in group III (26,73±1,469) in comparison with group II (p=0,001693).
Conclusion. Unhealthy mother lifestyle (sedentary lifestyle, smoking, drinking alcohol and eating foods containing tartrazine) leads to increasing apoptotic activity in organs of immune system (thymus and spleen) of children with the increasing of their age.

CORRELATION ANALYSIS OF THE FETO-PLACENTAL PARAMETERS ET INTRAUTERINE GROWTH RETARDATION

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Introduction/ Background

A number of studies suggest that some disproportions and retardations in feto-placental growth may lead to various pathologies in adults.

Aims

The aim of the present work was to identify alterations in correlations between placental and fetal parameters in cases of intrauterine growth restriction (IUGR) at 20-25 weeks of gestation (wg).

Methods

18 cases with IUGR and high placenta/fetal weight index (PFI) have been compared with 20 controls in cases of induced abortions for socio-economic reasons at 20-25 wg. Recorded data included weights of placenta (PW) and fetus (FW), fetus length (FL), head (Ch), chest (Cch), abdominal (Ca) circumferences, fetal kidneys (KW), liver (LW) and heart (HW), as well as some indices(FPI, FW/FL, LW/PW, HW/PW, KW/PW). The correlation analysis of the morphometric data was performed.

Results

IUGR group had smaller parameters of FW, FL, Cch, Ch, and Ca. All IUGR cases had decreased LW; KW and HW. PW was unchanged at 20-22 wg and increased at 23-25 wg, compared with controls. The negative correlation has been found between PW and LW/PW, FPI and LW at 20-22 wg, and between PW and HW/PW at 23-25 wg. The longer gestation the more new positive strong correlations have been discovered within the IUGR group (between FPI and HW, KW, LW, KW/PW).

Conclusions: the study suggests that the increase in the number and the intensity of correlations between morphometric parameters might represent a discrepancy between the fetal and placental growth at 20-25 wg and alterations in fetal organ adaptation mechanisms, which may result in future pathologies

MORPHOLOGICAL FEATURES OF PREGNANCY OF UTERINE LEIOMYOMAS

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Introduction/ Background

According to numerous publications of diseases among women of reproductive age, uterine leiomyoma are in the second place in the structure of gynecological pathology. Uterine leiomyoma - a benign, well-encapsulated tumor limitation, the source of which are the smooth muscle cells of the body of the uterus or cervix. According to different authors, in 0,4-3,9% of cases, the presence of tumor is diagnosed during pregnancy. Presence of myomas nodes leads to the threat of termination of pregnancy, missed abortion, the threat of fetal hypoxia, early and late gestosis, bleeding during pregnancy and childbirth . Moreover, the nature of complications depends on the size and morphological features of tumor's myoma nodes.

Aims

To study the morphological features of leiomyomas during pregnancy given the size of tumors and secondary changes in it.

Methods

Morphological examination of myomas was conducted in 25 cases of pregnancies completed caesarean section in 36-39 weeks of gestation with subsequent enucleation nodes. All myomas were divided into 2 groups: a core group of 10 nodes (more than 5 cm) and a control group of 15 nodes (less than 5 cm). On gross examination of tumors taken into consideration the number, size, texture, color, structure and the presence of degenerative changes. In each case of leyomioma nodes excised tissue portions from the central zone and the edge of the paracentral. The fragments of tissue were fixed in neutral formalin, followed by pouring into paraffin. With each produced histological preparations stained hematoxylin and eosin, and van Gieson's stain. Microscopic investigations were conducted on increasing × 100 × 400 with a microscope Primo Star (Carl Zeiss, Germany).

Results

At external examination of uterine control group were tightly-elastic and dense consistency. In the main group was uneven consistency of nodes, with the presence of foci of softening. In the main group tumor size reached 5.0 cm to 17.0 cm in diameter, these nodes along with portions of white or grayish white color observed small or large grayish yellow necrotic foci, and cystic formation was observed, and red degeneration, calcification. In the control group the size of myomas nodes were ranging from 0.5 cm to 4.5 cm in diameter, usually have multiple nodes localization. Grossly it had tumors pale pink, fibrous structure. Histological examination of the control group has some parts of the tumor that are rich in blood vessels and the cells that were in a state of hypertrophy, as observed focal stromal edema. While in the main group was characterized by not only the presence of necrosis, as well as defined areas of circulatory disorders, foci of hemorrhage, edema, or hyalinosis of tissue. This research showed a high variability of macroscopic and microscopic characteristics of leiomyomas and the presence of secondary changes of varying severity. There is every reason to believe that the size of the tumor nodes and pathologic features of leiomyomas may be an important marker for assessing the severity of disorders of homeostasis in utero-placental complex, and should be considered in the monitoring of pregnancy and childbirth.

MORPHOLOGICAL PATTERNS OF THE PLACENTA REMODELLING IN CASES OF IUGR AT 20-25 WEEKS OF GESTATION

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Introduction/ Background
Placental insufficiency is an important factor of the fetal intrauterine growth restriction (IUGR). .

Aims
To confirm the relation between placental morphology changes and fetal IUGR 20 placentas in cases of IUGR at 20-25 weeks of gestation (wg) were compared with 20 placentas at the same wg but without fetal IUGR

Methods
Material was divided: 10 placentas at 20-22wg and 10 - at 23-25wg both in cases of IUGR, which were studied with corresponding 10 (20-22wg) and 10 (23-25wg) controls. Volume fractions (vf) of the major placental components were determined from 5mmk paraffin sections.

Results
The results have shown that both placental and fetal weights in IUGR groups were smaller than in controls. The poor vascularisation and immaturity of the villous tree were found in cases of IUGR. The thickness of placental membrane increased from 10.74±0.09 at 20-22wg and 10.92±0.09 at 23-25wg in controls to 11.62±0.1 and 11.87±0.12mmk, $p<0.05$ correspondingly. The tendency of the decrease of fetal vessels vf and increase of the interstitium vf in cases of IUGR at 20-22wg has reached the significant level at 23-25 wg: correspondingly - 9.38±1.31 and 58.56±1.98 vs 14.38±2.48 and 52.07±2.75%, $p<0.05$ in controls.

Conclusions: the histopahological changes in placentas and the increased thickness of placental membrane might represent an important factor in the pathogenesis of the fetal intrauterine growth restriction.

AN INTEGRATED FRAMEWORK FOR HISTOLOGICAL IMAGE DATA ANALYTICS

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Introduction/ Background
Automated image analysis enables the mining of rich information from digitized histological slides. A major challenge is the complexity and large size of the images. Whole-slide images contain a multitude of different structures, like sections, regions of different tissue types and the contained cells. To make sense of these structures, often multiple analysis solutions must be combined. A common example is the initial identification of regions-of-interest and the subsequent evaluation of cellular structures with respect to these regions.

Aims
There is no general standard for representing image analysis data. Different analysis solutions may represent analysis data as either XML or JSON documents, spreadsheets or images. When combining multiple analysis solutions, the inconsistent data representation makes it necessary to convert information between different formats and to match related entities. This complicates data analytics considerably. To overcome this problem, we describe an integrated framework for histological image data analytics.

Methods
The framework represents image analysis data in an open relational data model. Image regions and cellular structures are represented as individual entities with properties and mutual relations. The framework incorporates multiple image analysis solutions for identifying image regions or cellular structures with machine-learning methods. The solutions are executed sequentially and populate the data model with more and more information from the image. Every step can take advantage of data generated in previous steps in order to target image processing operations to specific regions, or in order to reuse previously computed image features.

Results
The relational data model greatly simplifies data analytics in histological images. Region-specific statistics about cellular structures, or heat-maps of their spatial distribution can simply be computed by database queries. Furthermore, the relational data model enables the efficient management of the huge amounts of data generated by histological image analysis. We demonstrate the generic applicability of the framework by three example applications for the region-specific analysis of nuclear positivity, steatosis and inflammation in whole-slide images.

EXPERIMENTS IN EXTRACTING DIGITAL SLIDES FROM VIDEO

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Introduction/ Background

While digital pathology systems are becoming more and more affordable and thus diffused, for occasional, non diagnostic acquisitions (e.g., teaching) it might not be economically viable to buy a scanner. In addition to that, it is relatively common that videos from microscope are published for teaching purposes in particular in fields related to medical imaging [1]. Recently, a research group published a series of papers related to digital slide acquisition from non-automated microscopes [2]2 , [3]3. The developed technique allows to reconstruct a digital slide starting from a video flow acquired during slide examination. The proposed method seems interesting, and stimulated us in investigating further alternatives. In fact, the proposed process very closely resembles the creation of panoramas from multiple pictures through stitching, or the simulation of medium format photography through the Brenizer method [4] .

"Figure 1 - video acquisition setup"

Aims

Aim of the present work is to explore techniques for extracting digital slides from microscope video recordings, possibly using already available and open source software.

Methods

We searched for panorama stitching software and found a number of packages, including a widely used open source software (Hugin) [5] . We concentrated on it and on a commercial product (AutoPano). We designed a two steps experiment:
1) For a first approach, we decided to use a screencast of a digital slide. This to reduce the possible sources of error, and also to have a gold standard to compare with.
2) At a second step, we recorded video from a microscope (Olympus Provis AX70) using a commercial camera (Sony Nex-6) on an easily replicable hardware setup (fig.1).

We devised a sequence of steps to obtain digital slides as if they were panoramas recorded in video. This included pre-processing of the video source, and selection of parameters of the software for best reconstruction

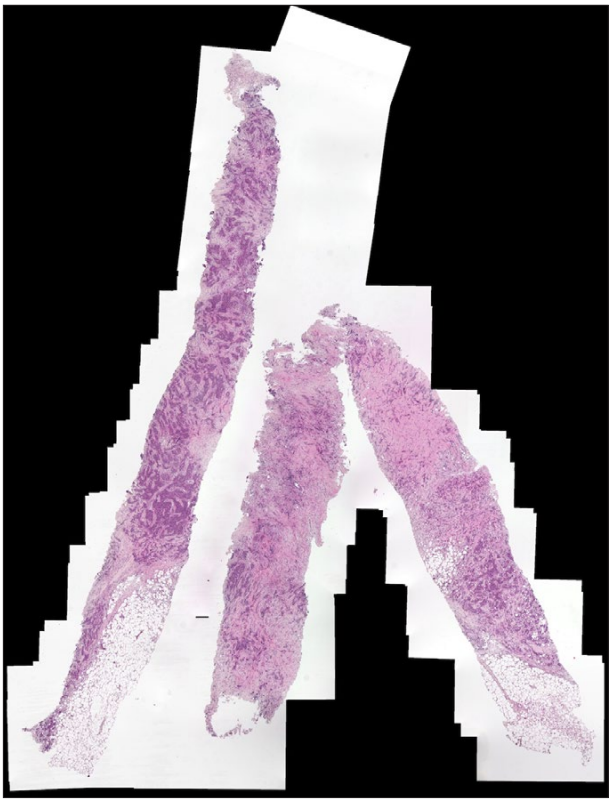
Results

The designed workflow include:

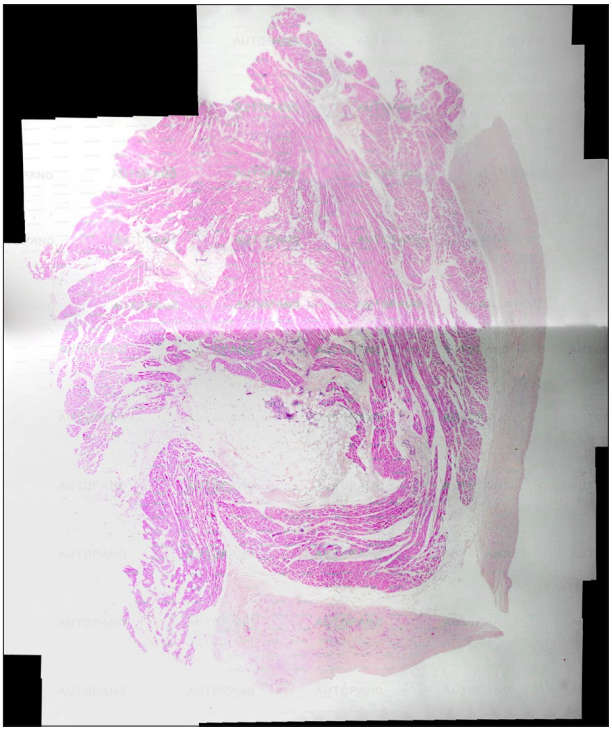
1. record a video from the microscope while browsing a case, taking care in covering the whole sample in a continuous way.
2. Transform the video in a sequence of images to feed the stitching software. This can be done with free software like ffmpeg and derivatives. It is not needed to have every frame of the video: the number is related to the speed of examination. In our experiment, 2-4 frames per second have been sufficient.
3. With Hugin (or AutoPano), open all the images and set them as if they were captured with a teleobjective with very long focal length (e.g., 1000mm). This to minimize abherrations in reconstruction.

Figure 1 shows a digital slide obtained with Hugin from digital microscope screencast; figure 2 shows a digital

slide obtained from a real microscope. The former is much better, and this indicates a possible way for occasional scanning.



"Figure 2 - digital slide from screen record"



"Figure 3 - digital slide from microscope record"

Open issues include:

- the method should be validated by comparing with a gold standard, like a digital slide of the sample;
- if the focus position changes during the video, focusing should occur and further processing could be needed for applying also focus stacking to the operations.
- While looking at a slide at higher magnifications, it is difficult to ensure to visit the whole sample during visualization. From this point of view, the realtime implementation presented in [2] allows for continuously checking what it has been acquired.
- This work has been partially founded by the EU FP7 program within the Marie Curie Project "AIDPATH", grant number 612471

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VIRTUAL MICROSCOPY NEW, OLD, OUT, OR JUST A PART OF EDUCATION

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Introduction/ Background

More than six years ago – accompanying the start of the new study program "Modellstudiengang" – we began a virtual microscopy program for our students. This started with slides to accompany the course to use during the lesson and for review of the slides at home (or in the library). But we wanted to go further.
How far have we come?

Aims

Our aim was to provide our students with more beneficial information and to increase the amount of material available - in the form of slides and accompanying exercises.
A secondary goal was streamlining the education of our students (fewer slides to manage and better opportunities for students to prepare for lessons).

Methods

The slides were scanned using NanoZoomer 2.0-HT slide scanner.
The virtual slides were made available to students using Slidebox system (version 4.4.3.) in three different ways:

1. We provided annotated slides with healthy (physiological) and diseased (pathological) samples to accompany the entire course. Some of the slides were for use during lessons, others as supplementary material.
2. The virtual slides were integrated into a new style of lecture ("blended learning") mixing learning opportunities from case reports, including clinical information; radiological images; and virtual slides. To review acquired knowledge memory-quizzes were used.
3. We complement the cases seen in the practical course "Autopsy – How, why, to what end?" with histology. Thus students are able to have a complete overview of the case from clinical history, to macroscopic findings and their correlation with the microscopic findings, to the final report.

Results

Usually the students were suprised, when they first come in contact with the virtual microscopy. But the Initial suprise yields to experimentation and getting used to it.

Virtual microscopy is not only just a part, but an important part of our education.

TELEPATHOLOGY A USEFUL METHOD IN THE DIAGNOSIS DIGESTIVE BIOPSIES WITH RARE ENTITIES. CASE PRESENTATION OF A COLLAGENOUS GASTRITIS

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Introduction/ Background

Collagenous gastritis is a rare entity in current surgical pathology practice. Since the introduction of the term until present only small series and isolated cases have been reported, generally by high-specialized centers in gastrointestinal pathology. In current practice, the diagnosis of this unusual affection is based on clinical data corroborated with endoscopic and pathological findings. Due to the small number of cases the diagnostic criteria are partly taken from microscopic colitis diagnosis. Thus, it is considered that the association of the appropriate clinical context (stomach pain, watery diarrhea, endoscopic nodular appearance) and histological gastritis changes with collagen deposition of dense material with a 10um minimum variable thickness that comprises capillaries and inflammatory elements. The technical advantage of a digital slide scanner is allowing access to an experienced pathologist in digestive pathology's opinion and conducting precision measurement.

Aims

Our presentation brings to attention of pathologist community a rarely diagnosed entity and the advantages of tele-pathology in timely accessing an expert opinion and performing accurate measurements.

Methods

We received gastric biopsy samples endoscopically taken from a 56-year female who complained of transit disorders, vomiting, epigastric pain that did not improve after IPP treatment. On endoscopy exam a nonspecific aspect with discrete erythema and vaguely nodular appearance of the gastric mucosae was noted. The specimens were paraffin embedded, and serial sections were stained HE, AB-PAS and MGG. The obtained slides were fully scanned and a gastrointestinal pathology expert advice was requested. Subsequently, a Mason's Trichrome stain and tenascin immunohistochemistry test were performed. The examined section showed gastric mucosal epithelium with scarce intraepithelial lymphocytes (5-6 lymphocytes/100 cells). The surface epithelium had the tendency to roll away from the variable thickened stroma. The glandular architecture and chorion were modified by a nonspecific inflammatory infiltrate. A diagnose of chronic inactive gastritis with intestinal metaplasia areas was propose, and an expert opinion was requested. Digitally scanned sections using the Leica II Aperio slide scanner were taken. Examination of slides was made using the Imagescope12.2 Leica. Micrometeric measurements of the hyaline material varied between 9.5-51um.

The review of glass slides on traditional optical microscope was made confirming the diagnosis.

Results

Collagenous gastritis remains an underestimated condition due to its low incidence and lack of expertise in the field. Hence telepathology becomes a useful tool for requesting a second opinion and precisions measurements.

THE USE OF SMARTPHONES AS ADJUVANT TOOL IN CERVICAL CYTOLOGIC DIAGNOSIS

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Introduction/ Background

Cervical cancer is still one of the world's deadliest forms of cancer for women, and the Pap smear remains the main screening test for prevention and early detection. Pap test is convenient and inexpensive, but, as was reported, there are variation in the sensitivity and specificity. Current smartphones allow the acquiring and transmission of static digital images, representing a cost efficient form of telepathology.

Aims

We report our experience in using smartphones and an instant messaging application to improve the cytologic diagnosis by teleconsultation.

Methods

The samples were conventional Pap smear and liquid base cytology slides. The images were taken by two of the authors, both junior pathologists, with the use of a Samsung Galaxy S3 Neo smartphone (8 MP, autofocus, Digital image stabilization) and a Zeiss PrimoStar microscope (415500-0057-000), 10x objective. We used the instant messaging application features to take the pictures and instantly send them to our fellow pathologist (if the images were resolution and focus suitable), then discuss the images in real time, using the chat options of the phone application. After reviewing the images together, and reaching to a conclusion, the images were submitted to an experienced pathologist in the same manner. They were reviewed a second time, and the results compared.

Results

The most common diagnosis we discussed on was ASC-US (19 cases), followed by L-SIL (6 cases) and NILM (5 cases). After we analysed and compared the data, we found that the time frame for diagnosis has improved. For the cases with divergent opinion we asked a third opinion to a colleague for the difficult cases. Consequently a senior pathologist review the glass slides and we compared the results with the digital diagnosis. The camera resolution was not limitative in this case, the used field power being 10x, thus the resolution and details were not impaired by the smartphone camera and instant messaging app limitations. Comparing our experience with the interobserver agreement on ASCUS and LSIL categories on glass slides we consider 'smartphone' pathology a useful tool. The use of smartphones allows junior pathologists to transmit Pap test consultations to senior pathologists and to ask for second opinions among fellow colleagues, improving costly effective the diagnosis accuracy.

AN INTEGRATED ENVIRONMENT FOR TISSUE MORPHOMETRICS AND ANALYTICS

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Introduction/ Background

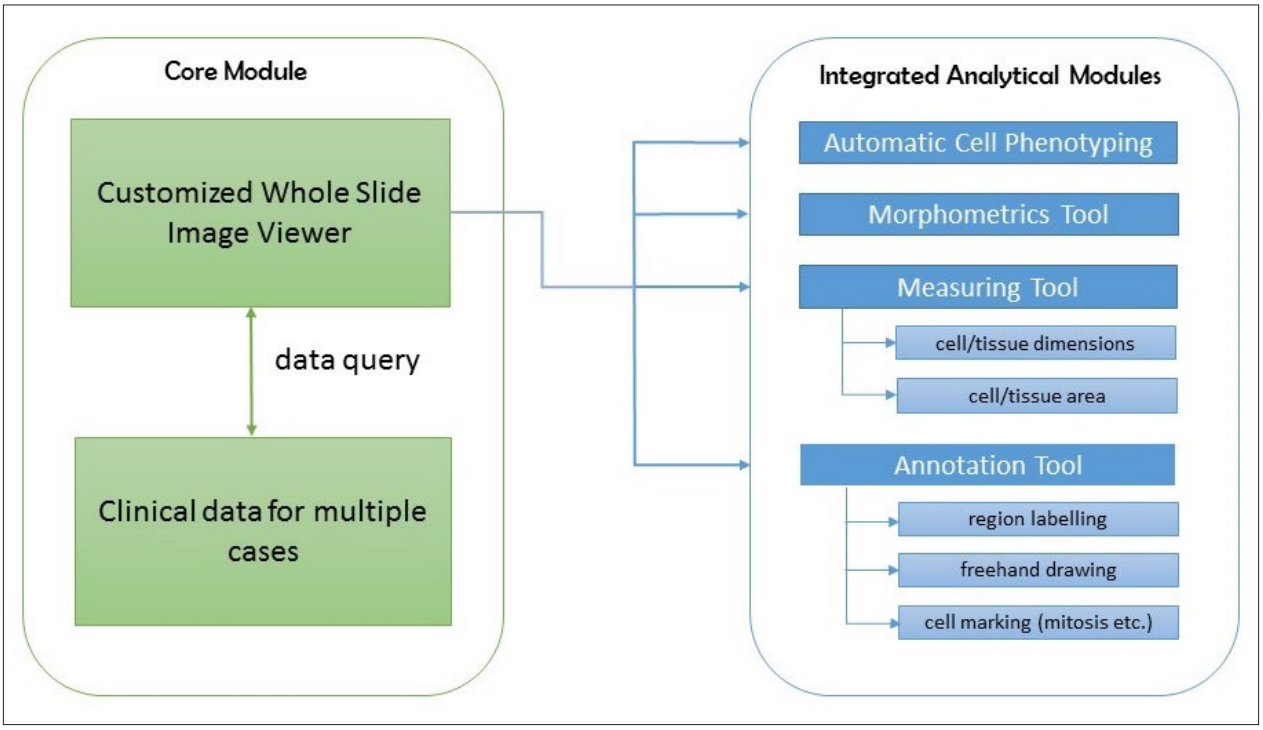
Attaining high reproducibility in cancer diagnosis is still one of the main challenges in modern pathology due to subjectivity [1] [2] [3]. An integrated framework to extract quantitative morphological features from histology images and perform analytics that can lead to the identification of outcome-related features will provide a more accurate and reproducible means to assess cancer [4] [5].

Aims

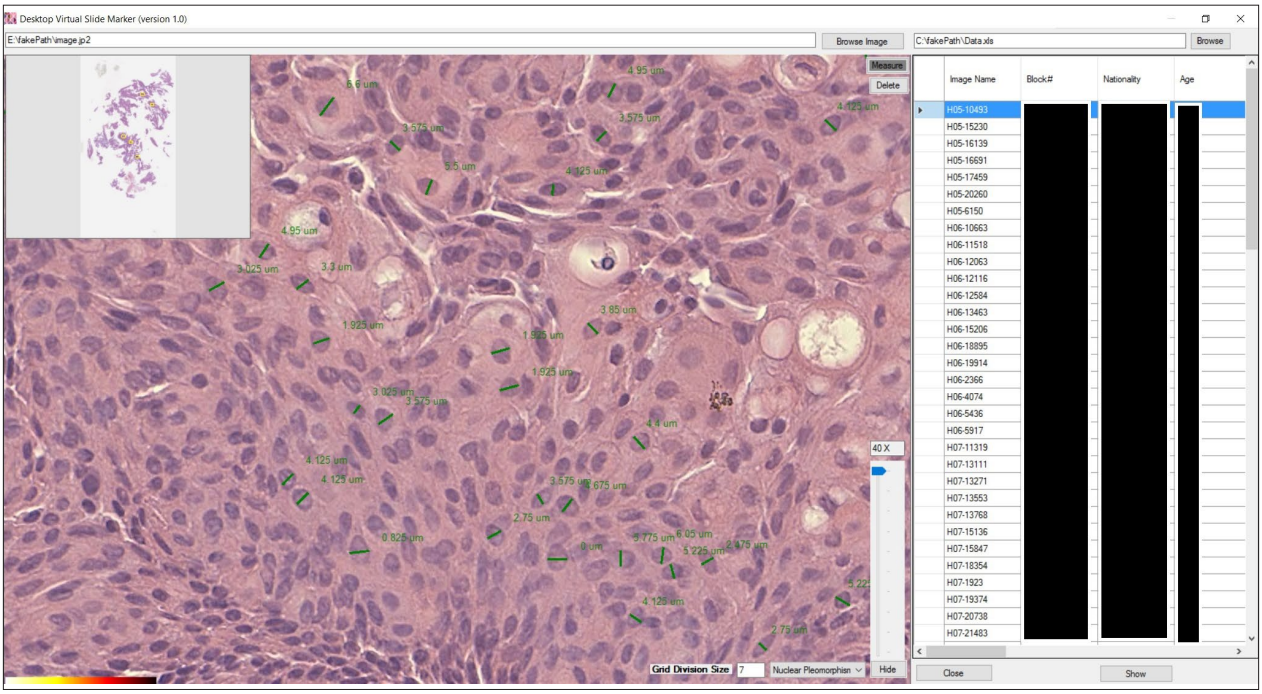
We propose an integrated environment which enables analytics of whole-slide tissue morphometry for a selected cohort of cancer patients. The proposed integrated environment includes three main components (Fig 1): (1) core module comprising of visualization of WSIs at multiple magnification levels, enabling display of clinical and imaging data for multiple cases simultaneously, (2) analytical module contains an interactive tool for measuring dimensions of tissue components and interactive annotation module (Fig 2), and (3) analytics module applied to data from a selected subset of cases or all the cases using quantitative morphological measurements including those derived from automatic phenotyping of cells [6] (Fig 3). The proposed environment can be further extended by adding new analytical modules and it can directly bring the benefits of quantitative analysis into pathological practices, thereby increasing reproducibility of cancer diagnosis. Furthermore, it can facilitate the studies of prognostic models, in which morphometric features strongly correlated with the outcome of cancers can be identified and used as image-based markers.

Methods

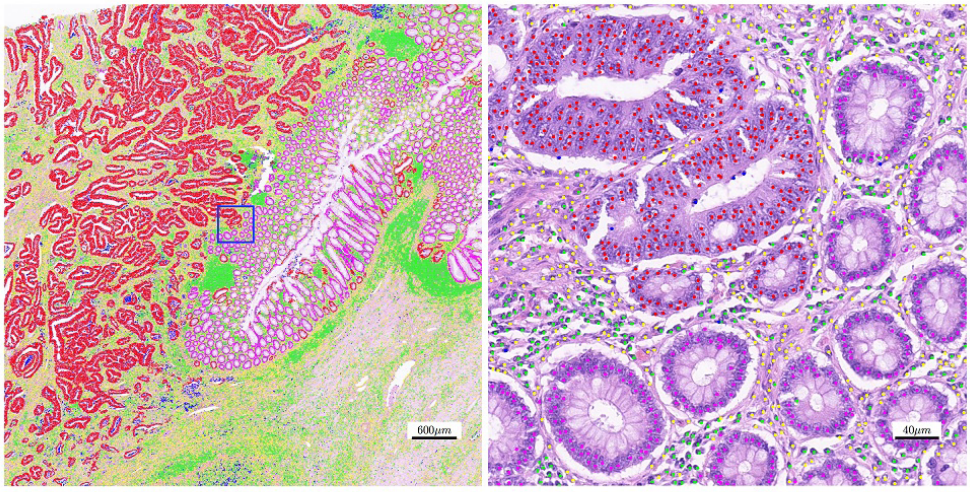
Our integrated environment for tissue analytics consists of core and analytical modules. The core module is a main portal to assess the already available imaging and clinical data, as well as analytical data which comes from integrated analytical modules.



"Fig. 1. Overview of functionality by core and example integrated modules"



"Fig. 2. An example of graded cancer region and cell measurements, right panel shows the clinical information for each case"



Nuclear Class	Precision	Recall	F1 score
Benign Epithelial Nucleus	0.902	0.922	0.912
Malignant Epithelial Nucleus	0.921	0.866	0.893
Inflammatory Nucleus	0.805	0.815	0.810
Fibroblast	0.689	0.756	0.721
Necrosis	0.603	0.649	0.625
Miscellaneous	0.372	0.346	0.358

"Fig 3. Results of cell phenotyping (a,b) Cells are phenotyped as as benign epithelial (magenta), malignant epithelial (red), inflammatory (green), fibroblast (yellow), necrosis (blue) (c) quantitative results for cell phenotyping."

These data are interconnected, allowing the users to query imaging data according to available variables in clinical and/or analytical data. It also enables the examination of the clinical and imaging data (Fig 2) at the same time. We developed a WSI viewer for exploring the tissue components at different magnification levels by supporting multi-threaded architecture for decompressing the image regions.

The state-of-the-art algorithm for automatic phenotyping of all cells in WSIs [6] is the analytic module that makes our interface different from other existing software. The algorithm is capable of identifying multiple classes of cells with high accuracy both in terms of quantitative (Fig 3a & 3b) and quantitative (Fig 3c) validation. This tool offers a fully automated analysis at the cell population level, in terms of the number and the spatial distribution of different cell types. This can, consequently reduce subjectivity and tediousness of the routine semiquantitative analyses performed by pathologists. This analytical tool is applicable to many prognostic applications such as identifying incidence of metastasis in sentinel lymph nodes, measuring the number of as well as locating tumor-infiltrating lymphocytes, etc.

Results

In this study, we have presented a fully customizable interactive environment for tissue analytics, equipped with measuring, annotating, and automatically cell phenotyping tools. The proposed integrated environment has remarkable potential to assist researchers and pathologists to reduce the human errors (if any) in diagnosing cancers. Further, this environment can serve as a benchmark to develop other morphometric measuring tools.

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SY06.02 | **Diagnostic Advances**

EVOLUTION AND REVOLUTION IN CONTEMPORARY PATHOLOGY

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Introduction/ Background
Cloud Pathology Group participated in the XI European Congress on Telepathology presenting the results of its great "in vitro research" about the use of HTS scanners in surgical pathology and in the XII European Congress on Digital Pathology presenting the preliminary results of its "in vivo research" done at the Busto Arsizio Multi-hospital Pathology Department, ended on June 30,2015.

Aims
The aim of this abstract is to complete the Paris presentation and to highlight the conclusions of the three years and a half research work on the application of digital pathology in surgical activities. These conclusions became the base of the CPG software prototype (digital suite) put on the market by the Company.

Methods
The methods applied during the "in vitro research" and the "in vivo research" were described in 2012 and 2014 presentations.
Afterwards, the work done consists in
- defining of specific tests to be executed on surgical specific diagnoses,
- defining the expected results of the tests,
- tests execution and validation of results,
- comparing expected and effective results,
- defining an evaluating the deviations
- defining how to proceed further.

This Loup method was applied n times until a definitive specific conclusion was found.

Results
There is no evidence that digital diagnoses present more risks than the microscopes' ones. The costs per diagnosis increase due to new investments and new running costs, however the level of the increase depends on the organizational model adopted. The higher is the volume of the slides treated, the lower are the costs per diagnosis. The higher is the standardization of the slides that the Laboratory produces, the higher is the accuracy of the diagnosis. The greater is the number of the pathologists involved in the network entitled to a diagnosis, the higher is the accuracy of the diagnosis (side effect of the specialization). The second opinion solution is a slow process; it is better to produce the diagnosis by involving more pathologists in the same process in the same time (collaborative diagnosis). The Data Base on work' tracking is the main requirement for the quality control procedure, for the evaluation process of the professional skills and for the work assignments. The electronic repository of the images instead of the physical stock of slides cuts relevant costs. The organisation of Data Base, its flexibility and its multi-proprietary approach are the keys of the quality of the entire solution. At all, digital pathology is a total change process. The fundamentals of its success are:
- clear comprehension of the organizational effects to be put on place,
- massive educational programs on new technologies and on change management, for pathologista, technicians and therapists,
- strong change management assistance,
- coherent behaviour in all people involved.

SEMI-AUTOMATIC CLASSIFICATION OF HISTOPATHOLOGICAL IMAGES: DEALING WITH INTER-SLIDE VARIATIONS

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Introduction/ Background
The large size and high resolution of histopathological whole slide images renders their manual annotation time-consuming and costly. State-of-the-art computer-based segmentation approaches are generally able to classify tissue reliably, but strong inter-slide variations between training and evaluation data can cause significant decreases in classification accuracy.

Aims
In this study, we focus on alpha-SMA stainings of the mouse kidney, and in particular on the classification of glomerular vs. non-glomerular regions. Even though all slides had been recorded using a common staining protocol, inter-slide variations could be observed. We investigate the impact of these variations as well as methods of resolution.

Methods
We propose an interactive, semi-automatic tissue classification approach [1] which adapts a pre-trained classification model to the new image on which classification should be performed. Image patches for which the class (glomerular/non-glomerular) is uncertain are automatically selected and presented to the user to determine the class label. The user interaction step is repeated several times to iteratively adjust the model to the characteristics of the new image. For image representation and classification, well known methods from the literature are utilized. Specifically, we combine Local Binary Patters with the support vector classifier.

Results
In case of 50 available labelled sample patches of a certain whole slide image, the overall classification rate increased from 92 % to 98 % through including the interactive labelling step. Even with only 20 labelled patches, accuracy already increased to 97 %. Without a pre-trained model, if training is performed on target domain data only, 88 % (20 labelled samples) and 95 % (50 labelled samples) accuracy, respectively, were obtained. If enough target domain data was available (about 20 images), the amount of source domain data was of minor relevance. The difference in outcome between a source domain training data set containing 100 patches from one whole slide image and a set containing 700 patches from seven images was lower than 1 %. Contrarily, without target domain data, the difference in accuracy was 10 % (82 % compared to 92 %) between these two settings. Execution runtime between two interaction steps is significantly below one second (0.23 s), which is an important usability criterion.

It proved to be beneficial to select specific target domain data in an active learning sense based on the currently available trained model. While experimental evaluation provided strong empirical evidence for increased classification performance with the proposed method, the additional manual effort can be kept at a low level. The labelling of e.g. 20 images per slide is surely less time consuming than the validation of a complete whole slide image processed with a fully automatic, but less reliable, segmentation approach. Finally, it should be highlighted that the proposed interaction protocol could easily be adapted to other histopathological classification or segmentation tasks, also for implementation in a clinical system.

ESTIMATING LIVER STEATOSIS: CAN ARTIFICIAL NEURAL NETWORK AND IMAGE ANALYSIS IMPROVE THE ACCURACY

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Introduction/ Background

Liver steatosis is very important in transplantation pathology as it directly influences the graft dysfunction. Pretransplant donor biopsy materials are evaluated by the pathologists, and degree of steatosis, especially large droplet steatosis (LDS) which is described as lipid droplets with a diameter of at least 15 micron, is estimated under light microscope. But when doing so, there can be great intra- and interobserver variability. In order to overcome this problem several automated systems and image analysis methods are used.

Aims

The most challenging issue for automated steatosis image analysis is to distinguish real oil droplets from sinusoidal regions. Although some morphometric features are employed to make this discrimination, whole feature space could not be represented for a fatty liver cell. In this study we have contributed a new approach, which tries to solve this discrimination issue with an artificial learning system.

Methods

Ten consecutive hematoxylin and eosin (HE) stained, formalin fixed paraffin embedded donor liver biopsies, reported by 2 pathologist, were evaluated by a third pathologist and steatosis percentage was given as total and LDS by using the percentage of area occupied by lipid droplets to total biopsy area. Automated image analysis was performed on about 200 photographs taken to represent the whole biopsy at X20 magnification by Zeiss Axio Scope.A1 microscope using a software Kameram™ and established as percentage of LDS to total biopsy area. Segmented positive (oil) and negative (non-oil) components are labeled by an expert pathologist, after some preprocesses they are fed to an Artificial Neural Network for training. We have used about 1000 droplets for training and 1500 droplets for performance evaluation. The proposed scheme is utilized to calculate liver fat ratio on digital images and the results are compared with expert's opinions.

Results

There was great variation among pathologists and when compared to the automated analysis and pathologists were prone to overestimate the steatosis (Table 1).

As this overestimation can lead to nonuse of the donor liver, the accurate assessment of the steatosis is critical. Since the biopsy is the gold standard for the assessment of steatosis, methodology of this examination should be as objective as possible. Our results show that automated assessment of liver steatosis is very useful in order not to loose donor livers, by overestimation. Automated image analysis used before were based on morphometric features of liver droplet regions, and use

Patient ID	System TDS	System LDS	Expert 1-2 TDS	Expert 1-2 LDS	Expert 3 TDS	Expert 3 LDS
1	8.8	5.5	30	25	25	15
2	6.5	2.6	15	10	35	5
3	8	6	25	20	35	30
4	6.1	3.7	13	8	15	5
5	4.3	1.9	10	8	25	5
6	5.1	2	8	3	25	
7	8.2	4.3	20	15	35	20
8	11.8	9.6	30	20	45	25
9	11	7.4	25	15	35	25

Table 1: The total droplet steatosis (TDS) and large droplet steatosis (LDS) percentages estimated by the pathologists and calculated by the software

of this Artificial Neural Network for training to discriminate sinusoidal areas from lipid areas reached a high accuracy. In this study, we proposed a new approach to discriminate lipid areas from sinusoids without using morphometric features, which needs be confirmed in a large cohort. The performance can be improved by employing some different pattern classification techniques such as Support Vector Machines as a future study

DIGITAL PATHOLOGY IN ITALY: PRELIMINARY RESULTS FROM A NATIONAL SURVEY

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Introduction/ Background

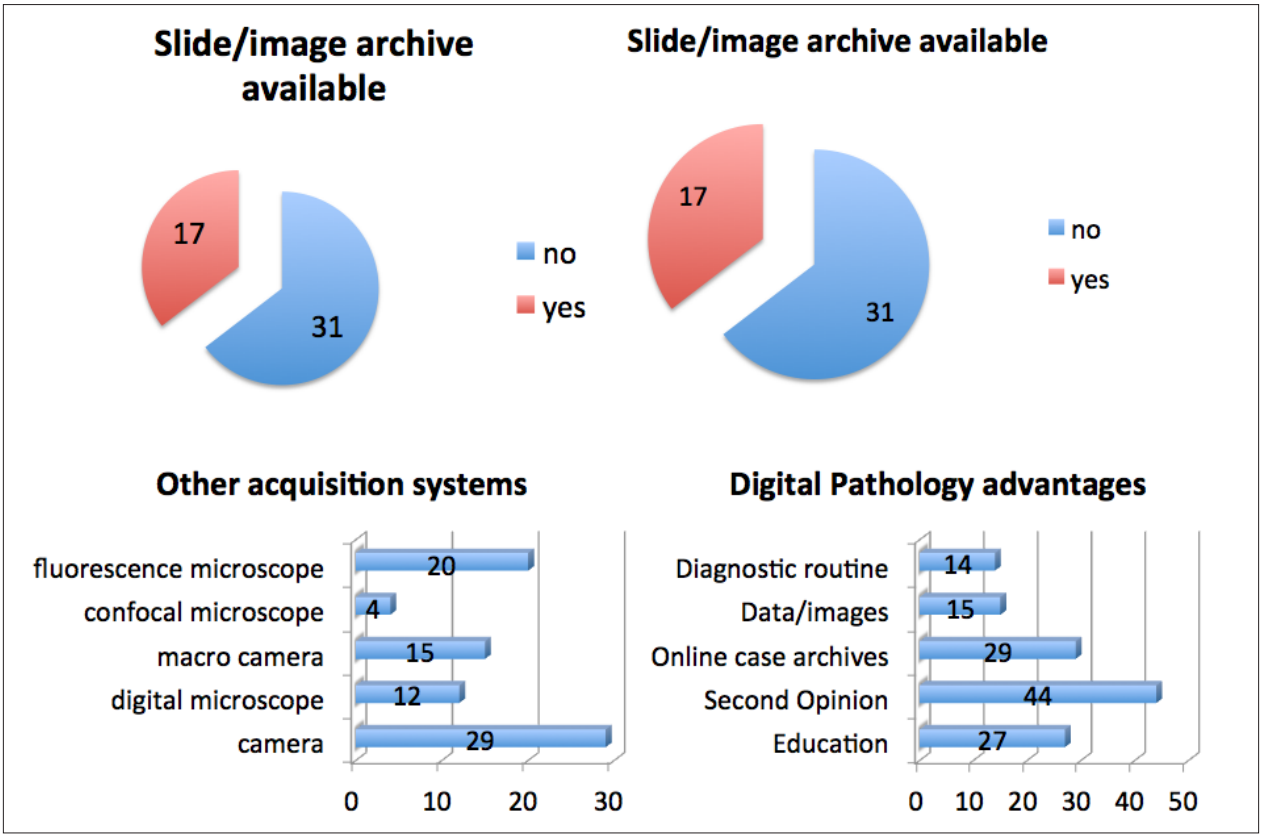
Digital pathology has been around since many years now, but it is still not fully integrated in work routine. The Italian Society of Pathology and CytoDiagnostics (SIAPEC) contributed to its development at national level, and is willing to stimulate better collaboration and sharing for the aims of continuing education and training. For this, a common infrastructure for hosting digital slides could be useful. For this, SIAPEC, in collaboration with the University of Udine, decided to investigate the current status of digital pathology in Italy, to foresee possible development paths.

Aims

The aim of the present work is to present preliminary results on digital pathology diffusion in the Italian pathology institutes, and to understand if there is interest and need for a common infrastructure for continuing education and training in pathology through digital slides.

Methods

A survey has been developed in two formats (online through Google Forms and as Word file) to ask 24 questions to Pathology laboratory Chairs. Recipients were contacted through email and addressed to both versions. The survey included questions on the availability of slide scanners, on the organization of slide/image archives (if any), on the aims of the archives, on the technical availability of slides and on their anonymization, and finally on the foreseen advantages of digital pathology. The survey started in January 2016.



"Fig.1 - A summary of findings"

Results

Of the 241 recipients (i.e., Pathology Institutes in Italy), 48 (20%) answered the survey at the time of submission. 14 Institutes (39%) declared to own at least one scanner. However, all the others have traditional tools for image acquisition: cameras on the microscope, macroscopic cameras, digital microscopes, fluorescence microscopes with acquisition tools, confocal/ deconvolution microscopes (for details see fig.1).

17 Institutes manage organized slide or image archives, however in all but 2 cases they are not integrated with the LIS. Who has an archive, typically stores 11-50 slides or images per month, with a total number of cases being between 500 and 1000. The most frequent aims of the archive are Education (7), Teleconsultation (6), Image analysis (6). Answers to technical questions (image formats, Z-axis scanning) were less frequent than others: details are not always known to pathologists. About half of the Institutes anonymize the slides by covering the tag, the others not. When asked about the possible advantages of digital pathology, Italian pathologists seems to be conservative. In fact, the most chosen application is second opinion (44), followed by online case development for scientific aims (29), and education (27). Diagnostic routine is chosen only 14 times, while the possibility of integration with other clinical data and images is chosen by 15 only. However, in general digital pathology seems not yet applied in diagnostic routine, but rather relegated to ancillary roles. This fact is also supported by the low number of high throughput scanners owned by Institutes: only 3 declared having large slide feeders (>100 slides). At the time of the congress updated results will be provided

INFLUENCE OF DISPLAY CHARACTERISTICS ON CLINICAL PERFORMANCE IN DIGITAL PATHOLOGY

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Introduction/ Background

Digital Pathology adoption is increasing rapidly. Recent technological advances have resulted in a steep increase in the performance and quality of digital pathology systems. Quality assurance mechanisms are being developed to ensure consistent quality of scanned slide images. However one important component that surprisingly is often overlooked is the display system. Pathologists base their diagnosis on the images presented by the display. The quality of these digital images depends on all of the components in the imaging chain, including the display itself. Even a perfectly scanned high quality image will not be useful if it is visualized on a low quality display.

Aims

The goal of this paper is to study important display characteristics and to determine what their effect is on percent correct diagnosis, reading time, diagnostic confidence and inter-pathologist-agreement. Furthermore a recommendation will be provided for minimum requirements of a digital pathology display system.

Methods

This paper combines and analyses results of several experiments that we have performed during the last two years. These studies included actual clinical studies where pathologists diagnose clinical images, reading studies where pathologists subjectively score quality of clinical images, as well as bench testing on both test and clinical images.

Separately analyzing the influence of display luminance, color settings, calibration and quality assurance, stability and resolution allows us to determine a relative importance of these characteristics. It also allows recommending minimal display specifications.

Percentage agreement between pathologists for: stable display, display with instable color, display with instable color and luminance

	Reader A	Reader B	two-reader aggregate
Non-aged	69.23 [53.6, 81.4]	55.26 [39.7, 69.9]	62.34 [51.2, 72.3]
Chroma aging	52.50 [37.5, 67.1]	50.00 [35.2, 64.8]	51.25 [40.5, 61.9]
Chroma + luma aging	50.00 [35.2, 64.8]	47.50 [32.9, 62.5]	48.75 [38.1, 59.5]

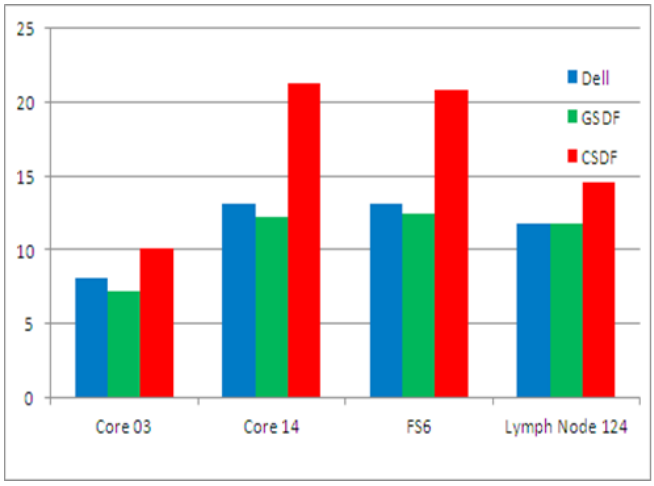
Average reading time for: stable display, display with instable color, display with instable color and luminance

	Reader A	Reader B
Non-aged	41.03 ± 5.38	30.79 ± 1.87
Chroma aging	49.13 ± 6.62	33.00 ± 2.13
Chroma + luma aging	51.20 ± 6.35	33.38 ± 2.26

Ease of reading for: stable display, display with instable color, display with instable color and luminance

	Reader A	Reader B
Non-aged	9.72 ± 0.19	9.74 ± 0.25
Chroma aging	8.83 ± 0.44	9.40 ± 0.31
Chroma + luma aging	8.38 ± 0.40	7.63 ± 0.59

"Influence of color and luminance variation on reading time, percentage agreeent and ease of reading"



Difference between feature foreground and background

Image	CSDF / GSDF	CSDF / sRGB
Core03	1.399	1.244
Core14	1.74	1.617
FS6	1.674	1.589
Lymph Note 124	1.24	1.239
	CSDF / GSDF	CSDF / sRGB
Average dE2000 difference between feature and background	1.513	1.422
Standard deviation of dE2000 difference between feature and background	0.235	0.209

"Influence of color settings on perceived contrast of clinically relevant features"

Results

A first clinical study analyzed the impact of luminance and color instability/aging of display systems on reading time, percent correct diagnosis, and inter pathologist agreement. 120 clinical digital pathology images were presented to pathologists. The images were scored and the diagnosis and reading time was recorded. The study shows that both luminance and color instability result into lower percent correct, lower inter pathologist agreement, and higher reading time. The results also suggest that color instability has a larger influence than luminance instability.

A second study focused on color settings of a display. Three different calibration settings were compared: "sRGB", "DICOM GSDF" and a recently proposed new standard "CSDF". Bench testing and subjective reader preference analysis was performed. Results indicate that perceived contrast of clinically relevant features in digital pathology images is higher when using CSDF compared to sRGB and DICOM GSDF. A final study looked at display size, resolution, contrast and luminance and their influence on subjective quality preference, ease of reading and reading time.

Based on the combination of these different results we make clear recommendations for minimum specifications for digital pathology display systems.

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OPTIMIZING OPTICAL AND DIGITAL RESOLUTION FOR BRIGHTFIELD WHOLE SLIDE SCANNING

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Introduction/ Background
Whole slide imaging lacks standards for defining image resolution. Scanner vendors usually describe the image resolution according to the microscope objectives "20X" or "40X". This has caused confusion, since scanning with a 20X objective lens may mean spatial resolution 0.25 to 0.5 microns per pixel, due to variable pixel size of the camera sensor and/or use of a magnifying relay lens.

Aims
Our aim was to compare image quality obtained by two digital cameras (with pixel sizes 5.5um and 3.1um conbined with 10X, 20X, and 40X Plan Apo objective lenses.

Methods
As for image quality readouts we used standardized resolution charts, Peak-Signal-To-Noise Ratio, and evaluation by three pathologists who ranked the images by their "visually lossless quality", when displayed with a 4K computer monitor. Lossless JPEG2000 was used as a reference.

Results
The differences in scanned image quality were significant. The image quality achieved with a CCD camera with 3.1 um pixels was superior in all tests. It was noteworthy that the camera with 3.1 um pixels (Lumenera 1265R) gave visually as good diagnostic image quality with 10X lens (0.31 um/pixel) as did the 5.5 um pixel camera (Lumenera LT425) with 20X lens (0.28 um/pixel). This gives a significant speed advantage in scanning of standard H&E slides, because the scanner needs to capture only one 10X field instead of four 20X fields. In higher resolution scanning tasks, such as cytology and in situ hybridization, we found 3.1 um pixel Lumenera 1265R camera and Plan-Apo 20X lens to give diagnostically satisfactory scanning results. We anticipate that in the future scanners will be equipped with cameras having small pixel size sensors (typically 2-3microns). This matches with the optical resolution of Plan-Apo objective lenses. Compared to current scanners, significant image quality and/or scanning speed improvement can be gained by upgrading the camera.

HEMATOXYLIN COUNTERSTAIN TO SIMPLIFY WHOLE SLIDE SCANNING OF IMMUNOFLUORESCENCE STAINS

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Introduction/ Background
Whole slide scanning of immunofluorescence (IF) stained slides is a well-recognized need, because fluorescence signal fading makes slides non-archivable. Technically scanning of IF has remained difficult, because tissue finding and autofocus operations are slow and prone to errors under epifluorescence illumination.

Aims
To simplify and to make IF scans more reliable, we started to look for counterstains that are compatible with IF and that can be scanned under brightfield illumination first.

Methods
Of the many alternative counterstains tested, light hematoxylin (H) proved the best counterstain for IF (with or without DAPI). Hematoxylin staining allowed the scanner (OIT Turboscanner) to perform rapid and accurate tissue finding and to define autofocus points, yet it did not increase background autofluorescence or decrease true fluorescence signal during subsequent scanning of IF under epifluorescence illumination. By using Cy2 (green) and Cy3 (orange) as secondary antibody fluorochromes (Jackson Immunotech), we were able to use standard xylene-based DPX mounting medium without observing significant fluorescence signal fading.

Results
As a result, IF scans of H counterstained slides could not be distinguished from those stained without hematoxylin counterstaining. As a bonus, the two scans (IF and H) could be viewed on the viewer screen side-by-side, or by gradually blending the two layers with each other. Both viewing modes were found useful in diagnostic dermatopathology when analyzing e.g. blistering skin lesions. A brightfield-IF scanning combination was also found useful in double immunostains, where cellular co-localization makes use of two precipitating (chromogenic) markers unreliable. The peroxidase IHC-hematoxylin-IF stain was exemplified with Ki-67 and pan-cytokeratin in breast cancer samples. The brightfield WSI of Ki-67 with H counterstain is an easily evaluable chromogenic DAB stain, but the image analysis software (ImmunoRatio2, embedded in the WSI viewer) utilizes the hidden pan-cytokeratin IF to create a cancer mask to exclude counting of non-epithelial stromal and lymphoid cells in the Ki-67 labeling index counting. Together these results demonstrate that a minor modification in the staining protocol (using hematoxylin instead of or in parallel to DAPI as counterstain) provides a significant help for whole slide scanning of immunofluorescence stains. Some scanners may need to be re-programmed to allow scanning of brightfield and epifluorescence automatically in sequence.

DIGITAL MICROSCOPY VERSUS CONVENTIONAL MICROSCOPY IN ASSESSMENT OF JEJUNE INTRAEPITHELIAL LYMPHOCYTES

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Introduction/ Background

Leukocytes infiltrates assessment in the lining epithelium of the digestive tract is a common request in current pathology practice both in terms of diagnosis and quantification of the therapeutic response. Frequently analyzed are lymphocytes positive for CD4 and CD8 in the context of gluten sensitive enteropathy. Microscopic quantification remains a time consuming and a laborious work. The possibility of human error puts at question the accuracy of data provided. Implementation of digital image acquisition techniques brings a relative comfort in delimitation the areas of interest, quantifying those areas and introduces digital algorithms that can determine the number of positive signals on the area of interest.

Aims

Establishing quantitative differences between the data acquired using optical microscopy versus digital evaluation of intraepithelial leukocytes (CD3+, CD4+, CD8+).

Methods

We analyzed paraffin embedded jejune segments fixed in buffered formalin (10%), taken from healthy patients (n= 15), monitoring the positive lymphocyte count and CD4 + CD8 + ratio. From each block, 4 sections of 3 µm thickness were taken. One section was stained with hematoxylin and eosin, and the other 3 sections were stained immunohistochemically using three steps-indirect streptavidin method for anti CD3 monoclonal antibodies, anti CD4 and CD8. The sections were referred to five independent pathologists in order to quantify positive leukocyte number in 100 enterocytes using optical microscopy and subsequent transmission microscopy images projected on high-definition monitors. Readings were performed on optical microscopes Leica DM750 objective 10X 40X eyepieces FN20. Image acquisition was made with 5-megapixel Leica microscope camera, ICC50 model, in direct transmission (stream) USB 2.0, monitors Dell 27 "HDMI IPS. For normalization and comparison of data, reporting was done in an area of 1 mm2. The variation between observers for the two methods were calculated (k).

Results

Interobserver variability for CD3 in optical was estimated as slight agreement (k0.36) in traditional microscopy compared to substantial agreement for digital assessment (0.68).

For CD4 and CD8 markers the differences were less significant, substantial agreement in both categories (CD4 0.72 for optical evaluation vs 0.78 in digital evaluation data and for CD8 0.65 vs 0.72 respectively).

In our opinion the use of digital image acquisition techniques in quantification of intraepithelial leukocytes increases concordance degree among observers through diminishing the subjective factors and by choosing identical ROIs.

MICROANATOMICAL ANALYSIS AND QUANTIFICATION OF PLASMA CELL NICHE INTERACTIONS IN THE BONE MARROW

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Long-lived plasma cells (PCs), responsible for the production of long-term antibody titers, have been shown to survive in the bone marrow for months to years in the absence of antigen. They are supported by a special microenvironment, the PC survival niche. Various cell types have been reported to contribute to this niche by providing survival factors, e.g. CXCL12-producing reticular stromal cells. Additionally, hematopoietic cells have been shown to mediate PC survival in vivo, amongst them megakaryocytes and eosinophils, but the spatio-temporal dynamics of the various niche components in the tissue remain elusive.

The aim of our work is to analyze the cellular and molecular composition of plasma cell survival niches in the bone marrow in situ.

In order to unambiguously quantify the localization of PCs, we are analyzing bone marrow cryosections and whole mounts for PCs, stromal cells, vasculature and accessory niche cells. Additionally, we have developed a computer modeling approach which allows us to distinguish random co-localization from non-random cell positioning.

Using these approaches, we have previously shown that PCs directly contact reticular stromal cells in a non-random fashion, while 30% of PCs are found in 10 µm vicinity to eosinophils, which represent only transient contributors to the niche. We have now analyzed PC localization in relation to mineralized bone, bone marrow vasculature and hematopoietic cell types in 3 dimensions and found that PC niches are situated at large distance to sinusoids.

Semi-automated 3D analyses of whole mounts allow for a comprehensive and unbiased quantification of PC localization and their possible interactions with accessory niche cells in the bone marrow. We show that the survival niche for long-lived PCs is located distant from sinusoidal blood vessels, in contrast to what has been reported for the hematopoietic stem cell niche. We are now testing ways to mobilize PCs from their niches, which should result in shifted PC localization - from vessel-distant to peri-sinusoidal spaces. We are further exploring ways to perform multiplexed histological analysis using multi-epitope ligand cartography (MELC) in the bone marrow in order to further characterize the plasma cell niche.

THE REPRODUCIBILITY INDEX OF PATHOLOGICAL DIAGNOSIS AND RARE CASES. THE RESULTS OF THE ON-LINE DIAGNOSTIC COMPETITION "FINAL DIAGNOSIS".

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Introduction/ Background

UNIM Ltd. have created the SAAS platform DPathology that can be used for saving and studying histological slides and it doesn't require an installation of a special software. You can use the platform with all the modern internet browsers. The SAAS platform gives all the specialists a chance to analyze remotely digital histological slides. It increases the accuracy of diagnostics and speeds up the medical assessment

Aims

1. To indicate the importance of collecting rare cases and expert assessment via digital microscopy
2. Using the Digital Pathology© platform to carry out educational and competitive diagnostic measures.

Methods

Fourteen rare cases from different sub-specializations field in pathology were selected by UNIM LTD with expert's pathologists from the Czech Republic and Italy and additionally validated in Norway and the UK (blind method). The slides were digitized and introduced with clinical information to 250 specialists registered to take part in the competition "Final diagnosis"©.

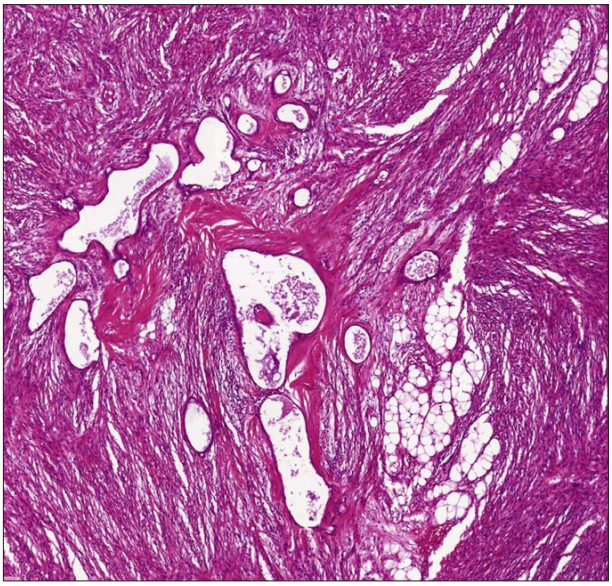
Results

The range of the totally correct answers varies between 3 and 56 percent. The most difficult case for the participants was the one with no tumorous pathology: ectopic hamartomatous thymoma [[1]1] There were 3 percent of full match

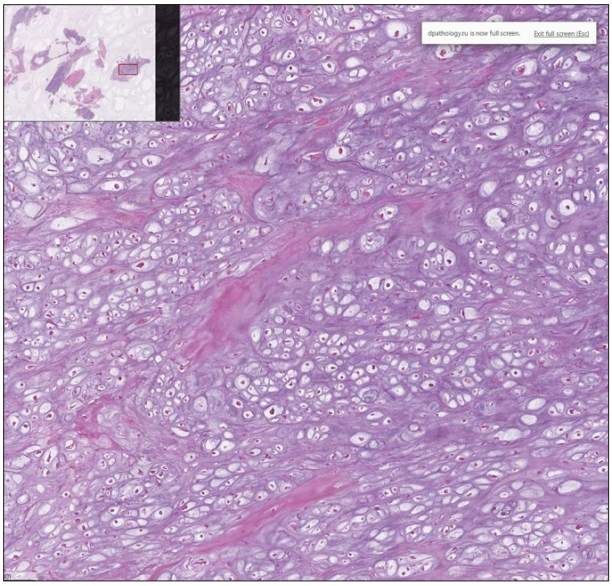
The biggest number of full match to experts' diagnoses can be seen in the case: Grade 2 central chondrosarcoma with 72% of agreement

To analyze the disagreements we divided them in two groups:

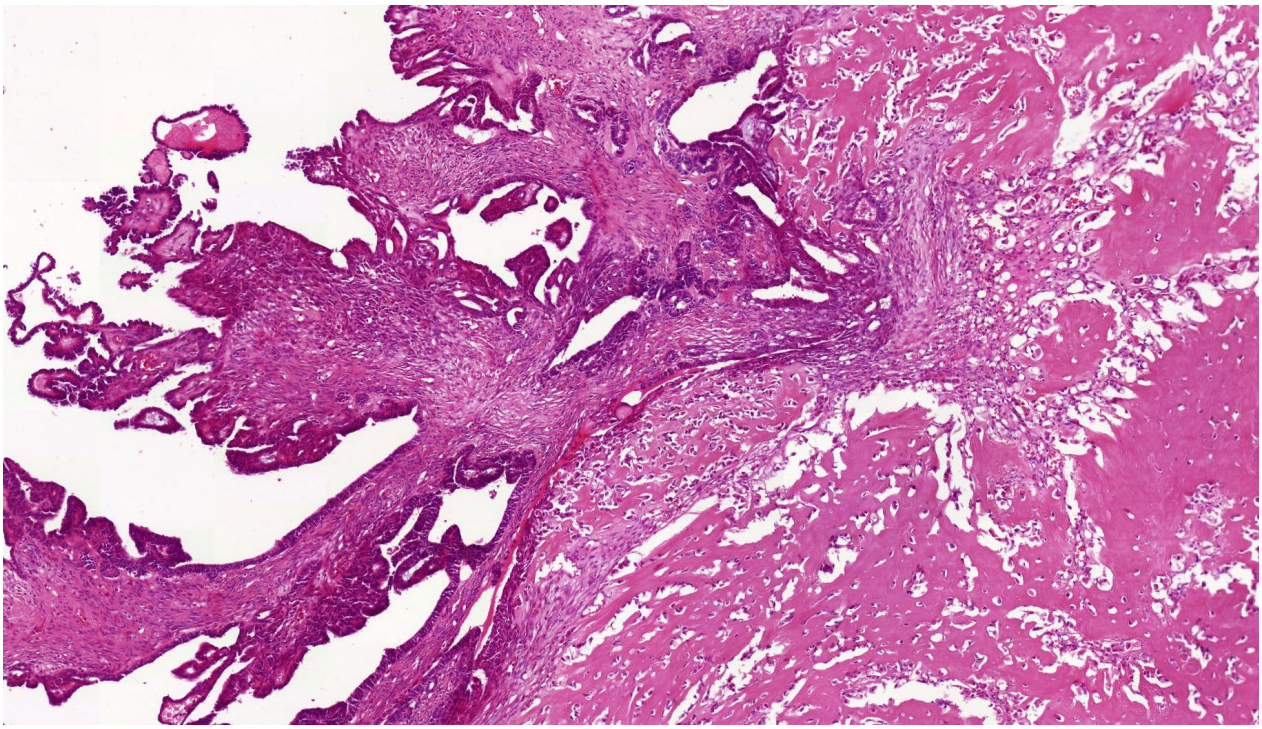
Mayor disagreement – potentially not correct histological diagnosis will change the clinical tactics of patient's treatment (considering the malignant pathology as a benign pathology, considering the benign pathology as a malignant, changing the stage of disease). Potentially wrong pathological diagnosis leads to wrong course



"ectopic hamartomatous thymoma"



"central chondrosarcoma"



"hyalinized endometrioid adenocarcinoma"

of patient's treatment and wrong chemotherapy, etc. Minor disagreement – potentially incorrect diagnosis doesn't have any clinical matter. This tactic showed that the case of hyalinized endometrioid adenocarcinoma [[2]2] turned out to be the most difficult one for the participants. The range of mayor disagreement here was 66 percent, mostly because cases was interpret as carcinosarcoma (63/93), while the agreement is 14 percent

And myxoinflammatory fibroblastic sarcoma of soft tissues [[3]3], with major disagreement in 67.4% (pict case 10).

All the data is shown in table 1.

Agreement, % Minor disagreement, % Major disagreement, %
case1, N=97, Teratocarcinosarcoma of the nasal cavity [[4]4] 0,12 0,61 0,26
case 2, N=89 Juxtaoral organ of Chievitz [[5]5] 0,13 0,50 0,37
case3, N=93, Mammary Analogue Secretory Carcinoma of Salivary Glands, Containing the ETV6-NTRK3 Fusion Gene [[6]6] 0,365 0,48 0,15
case 4, N=96 Low-grade sebaceous carcinoma of the skin [[7]7] 0,10 0,68 0,23
case 5, N=99 t(6;11) translocation carcinoma (Rosette-forming tumor of the kidney) [[8]8] 0,31 0,35 0,32
case 6, N=93 Hyalinized endometrioid adenocarcinoma [[9]9] 0,14 0,20 0,67
case 7, N=96 Ectopic hamartomatous thymoma 0,03 0,82 0,15
case 8, N=91 Prolapse of the fallopian tube after hysterectomy 0,16 0,67 0,14
case 9, N=87 Phosphaturic mesenchymal tumor of soft tissues with calcification 0,23 0,34 0,33
case 10, N=92 Myxoinflammatory fibroblastic sarcoma of soft tissues [3] 0,13 0,20 0,67
case 11, N=87 Enchondroma 0,35 0,15 0,49
case12, N=88 Atypical chondromatous tumor/ Grade 1 chondrosarcoma 0,20 0,42 0,375
case 13, N=87 Grade 2 central chondrosarcoma 0,72 0,07 0,21

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PATHOLOGY ASSISTANT (C) - GAMECHANGER OF PATHOLOGY DIAGNOSTIC

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Introduction/ Background

UNIM Ltd. specializes in pathology diagnostics and digital pathology. We have created the SAAS platform DPathology that can be used for saving and studying histological slides and it doesn't require an installation of a special software. You can use the platform with all the modern internet browsers. The SAAS platform gives all the specialists a chance to analyze remotely digital histological slides. It increases the accuracy of diagnostics and speeds up the medical assessment. The platform is used in several hospitals to get a consultation from the leading specialists from all over the world. It also can be used to create the Medical Big Data and the database for pathologists.

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Aims

Using our platform, we developed a digital multi-head microscope system for the professional society and we are planning on holding the first education program based on it on the 15th of January 2016. Specialists from Czech Republic will be our speakers and as the first round we want to show four cases of skin cancer.

Methods

This year we hold a competition for pathologists based on Dpathology software (www.finaldiagnoses.ru). 250 specialists registered to take part in the competition. There were fourteen cases from different specializations: we provided participants with clinical data and digitized histological slides. We got the cases from our partners from the Czech Republic and Italy.

Results

When the competition ended, we received many favorable reviews and we decided to start another project a little bit similar to the competition. Every month we show three interesting and difficult to diagnose cases provided by the leading Russian pathologists. The participants can look through the clinical data and digitized histological slides, and then discuss what they see among their professional society. There are 400 specialists from post USSR countries. Moreover, we get a few proposal of partnership to start a similar project in EU. And the last product in line is Pathology Assistant. It is a game changer. Pathology Assistant is a Digital Pathology©technology driven application for pathology diagnostics, tool to innovate pathology diagnostics in more simple, proven by analytical algorithm, automatically delivering anticipated support way. The service provides vast and structured database of validated cases, intuitive interface, fast and convenient system of analytical search. Pathology Assistant will streamline and simplify pathologist's way to the right decision. Pathologists from Memorial Sloan Catering and biggest EU labs are working on preparing the content for the project.

OPEN SOURCE SYSTEMS IN DIGITAL PATHOLOGY

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Introduction/ Background
Digital pathology is rapidly becoming popular worldwide. It not only helps to understand the nature of the disease through the analysis of tissue-based images but also advances our statistical knowledge of the disease-related factors via high throughput analysis of the imaging data. Such analysis can be done by using either commercial or open source systems.

Aims
Generally in comparison to commercial software and depends upon the scientific question to be answered, implementation of open source systems tend to be more challenging. However, unlike commercial software, open source systems are free with a high level of flexibilities in their functionalities which in turn makes them suitable for designing complex image processing systems as well as performing massive data analysis.

Methods
Here I review OpenCV (Open Source Computer Vision), one of the most powerful library of programming functions mainly aimed at real-time computer vision and its application in Digital Pathology. I also briefly introduce the Image Segmentation & Registration (ITK) library which is another open-source, cross-platform system with extensive suite of software tools for image analysis. Finally I give an overview of two other open source software, i.e. Aladin and Topcat , developed mainly for astronomical applications, and their usage in Digital Pathology.

Results
Open source systems/software provide researcher with an alternative method to successfully address complex problems in Digital Pathology.

ACCURACY OF WHOLE SLIDE IMAGING STACK ALIGNMENT IN CONSECUTIVE SECTIONS OF THE CAROTID ARTERY

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Introduction/ Background

Atherosclerosis is a chronic inflammatory disease of middle-sized and large arteries, characterized by the accumulation of inflammatory cells, especially macrophages [1] . A detailed visualization of the presence and distribution of macrophages in the atherosclerotic plaque contributes to a better understanding of the pathogenesis of atherosclerosis and the onset of acute coronary syndromes after atherosclerotic plaque rupture. Three-dimensional (3D) reconstruction of histology sections has the potential to improve both the detection of lesions as well as understanding in plaque growth and destabilization.

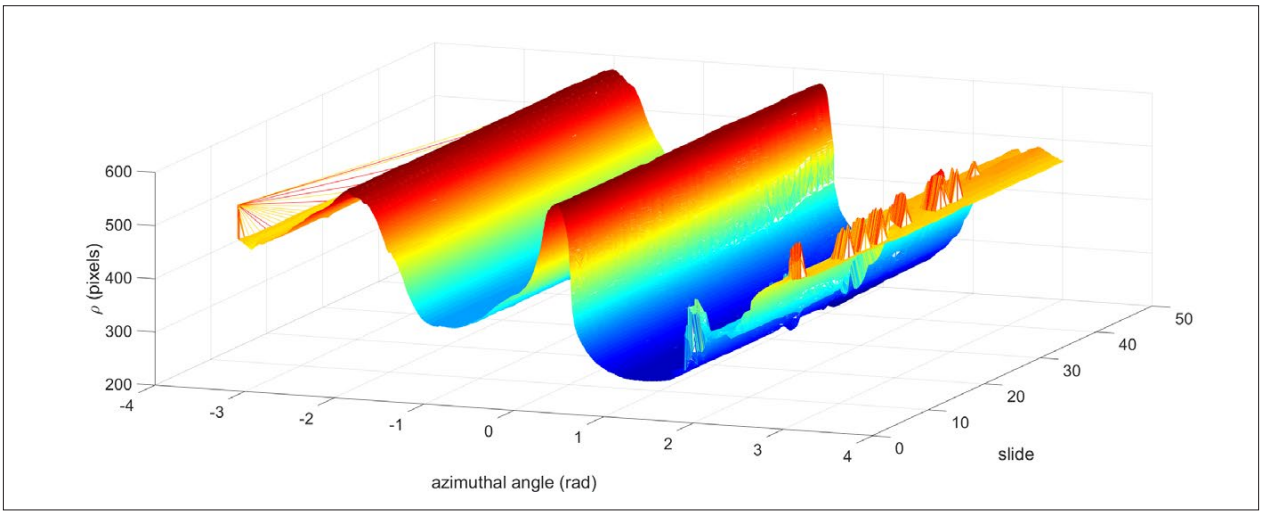
Aims

The objective of this study is to implement a image marker independent 3D histology reconstruction method in order to visualize the arteriosclerotic vessel and evaluate its accuracy.

Methods

A dataset comprising 48 consecutive cross-sections with a slice thickness of 10µm of a formalin-fixed paraffin-embedded (FFPE) carotid artery was used. The slides were double stained with monoclonal antibodies and were scanned with an Olympus dotSlide scanner with a 10x objective leading to 0.65 micron pixel size. In these images, the smooth muscle cells and macrophages were visualized in blue and red, respectively. Rigid, rigid & affine, and rigid & affine & b-spline (non-rigid) automatic stack alignment was performed using elastix, an open-source toolbox for alignment of images [2]. As a consequence of the image deformation in non-rigid approaches, the diagnostic accuracy might be hindered. Therefore a small bending energy, i.e., sum of the spatial second-order derivatives of the transformation, was allowed. In order to increase processing speed, the stack alignment was performed on downsampled data.

An automatically determined mask of the vessel was used for pair-wise reconstruction of the vessel with respect to the middle slide that was chosen as a reference section. Accuracy was visually assessed using a surface plot of the lumen of the vessel. In addition, the Dice similarity coefficient, which is a measure of spatial image overlap, of consecutive pairs of slides was calculated for the different stack alignment approaches.



"Color surface plot of the vessel lumen in cylindrical coordinates."

Results

Visual assessment of the surface plot of the vessels' lumen after pair-wise stack alignment, showed a relatively smooth surface of the lumen. This was the case for the rigid (i.e. translation and rotation), rigid & affine, and rigid & affine & b-spline approaches.

The Dice similarity coefficient of the registered masks increased with each additional alignment step. Slides alignment using rigid, rigid & affine and rigid & affine & b-spline approaches resulted in average Dice similarity coefficient of 0.85, 0.87, and 0.98, respectively. A more accurate result of the alignment comes at the cost of an increase in computation time by roughly a factor of two in each additional alignment step.

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ASSESSMENT OF MICROVASCULAR DENSITY IN THE NEUROENDOCRINE TUMORS OF THE PANCREAS: A CORRELATION WITH MULTIDETECTOR COMPUTED TOMOGRAPHY (MDCT) FEATURES AND TUMOR GRADE.

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Introduction/ Background

The biologic course of pancreatic neuroendocrine tumors (NET) can be accurately predicted on the basis of pathology parameters which, unfortunately, cannot be fully assessed until the entire tumor has been resected. When obvious landmarks of malignancy (metastases, vascular invasion) are lacking, tumour size is the only reliable preoperative parameter used to differentiate between presumably benign and malignant pancreatic NETs. It is generally accepted that pancreatic NETs exceeding 2 cm in their diameter may have borderline pathology or even malignancy. Anyway, up to 10% of tumors with diameter less than 2 cm are although malignant. That is why there is need in studies focusing on additional imaging characteristics, such as contrast-enhanced MDCT, useful for the aggressiveness assessment.

Aims

To determine whether it is possible to predict pancreatic NET Grade and microvascular density (MVD) according to contrast-enhanced MDCT findings.

Methods

A retrospective study was conducted and 70 patients with pancreatic NETs were retrieved from the Institutional archives. Twenty eight of them had met the including criteria (a - patients underwent surgery in our Institution and b - patients underwent preoperative dynamic abdominal CT within 30 days prior surgery) and comprised the study population. Thus, research included 15 patients with functioning and non-functioning pancreatic NETs Grade 1 and 13 of patients with Grade 2 (according to the WHO classification of 2010).

Radiology

All preoperative CT examinations were performed by a 256-slice CT scanner. Dynamic CT images, including non-enhanced, arterial, portal venous and delay phase images, were obtained for all patients. For contrast-enhanced CT, 90–120 mL of high concentration contrast media - iomeprol 400 (Iomeron 400; Bracco Imaging SpA, Milan, Italy). CT images were reconstructed with a section thickness of 1-2 mm.

Pathology

For identification of vessels, regardless lymphatic or venous, immunohistochemistry with CD34 (clone QBEnd/10, CellMarque) was performed on formalin-fixed paraffin-embedded (FFPE) tumor blocks.

Image analysis

Density of vessels was defined on 3 fields at x 200 (~ 1,8 mm2). The choice of fields of vision was carried out among sites with visually greatest density of vessels ("hot spots"). Determined total number of the pixels belonging to vessels by the Color Threshold tool (ImageJ) and divided this number into total of pixels in the image.

Results

The median of density of vessels at patients with NET Grade 1 made 8% ± 2% whereas in group of patients with NET Grade 2 this indicator made 5 ± 4%. The difference was statistically significant ($p < 0,05$). Mean arterial enhancement ratio was 1.66(0.42) in Grade 1 and 1.04(0.39) in Grade 2 pancreatic NET ($p<0.01$); and correlated with intratumoral MVD ($r=0.61$, $p<0.005$) and tumor grade ($p<0.01$). Vascular invasion, metastases and pancreatic duct dilatation were observed in two cases, both in G2 tumors. Arterial enhancement ratio <1.1, size≥20 mm, ill-defined borders and tumor non-homogeneity showed 83%, 74%, 70% and 56% accuracy in diagnosing G2 tumor respectively, while the accuracy of two of these criteria used in combination was 91%. Contrast-enhanced MDCT-features of pancreatic NET correlate with MVD and can predict tumor grade during preoperative staging.

AUTOMATED QUANTIFICATION OF PROLIFERATION WITH AUTOMATED HOT-SPOT SELECTION IN PHOSPHOHISTONE H3/MART1 DUAL-STAINED MELANOMAS

P.S. Nielsen*[1], R. Riber-Hansen[1], H. Schmidt[2], T. Steiniche[1]

[1]Aarhus University Hospital, Department of Pathology, Aarhus, Denmark, [2]Aarhus University Hospital, Department of Oncology, Aarhus, Denmark

Introduction/ Background

Staging of melanoma includes quantification of a proliferation index, i.e., presumed melanocytic mitoses of H&E stains are counted manually in hot spots. Yet, its reproducibility and prognostic impact increases by immunohistochemical dual staining for phosphohistone H3 (PHH3) and MART1, which also may enable fully automated quantification by image analysis.

Aims

To ensure manageable workloads and repeatable measurements in modern pathology, the study aimed to present an automated quantification of proliferation with automated hot-spot selection in PHH3/MART1-stained melanomas.

Methods

Formalin-fixed, paraffin-embedded tissue from 153 consecutive stage I/II melanoma patients was immunohistochemically dual-stained for PHH3 and MART1, and whole slide images were captured. An algorithm that automatically detects the number of PHH3/MART1-positive cells was developed using commercial software. In preprocessing, the intensity band of the HSI color model and color deconvolution including a standard deviation filter highlighted PHH3 positivity. Thresholding functions classified the image into brown PHH3, red MART1, and blue hematoxylin. Finally, postprocessing algorithms pinpointed PHH3/MART1-positive cells based on size, MART1 surrounding, color intensity, and nuclear irregularity. Based on the labels of image analysis, a hot spot was automatically selected by a processing step where circles that detect PHH3/MART1-positive cells produce a heat map according to their cluster. The number of PHH3/MART1-positive cells was counted both automatically and manually in the global tumor area and in a manually and automatically selected hot spot, i.e., a fixed 1-mm^2 square.

Results

The mean difference between manual and automated global counts was 2.9 cells/mm^2 ($P = 0.0071$) and 0.23 cells per hot spot ($P = 0.96$) for automated counts in manually and automatically selected hot spots. In 77% of cases, manual and automated hot spots overlapped. Fully manual hot-spot counts yielded the highest prognostic performance with an adjusted hazard ratio of 5.5 (95% CI, 1.3–24, $P = 0.024$) as opposed to 1.3 (95% CI, 0.61–2.9, $P = 0.47$) for automated counts with automated hot spots. In ***conclusion,*** the automated index and automated hot-spot selection were highly correlated to their manual counterpart, but altogether their prognostic impact was noticeably reduced. Because correct recognition of only one PHH3/MART1-positive cell seems important, extremely high sensitivity and specificity of the algorithm is required for prognostic purposes. The automated analysis may thus still aid and improve the pathologists' detection of mitoses in melanoma and possibly be useful in other malignancies and future research studies.

APPLICATION OF KI-67 ANALYSIS IN A DISTRIBUTED COMPUTING INFRASTRUCTURE

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Introduction/ Background

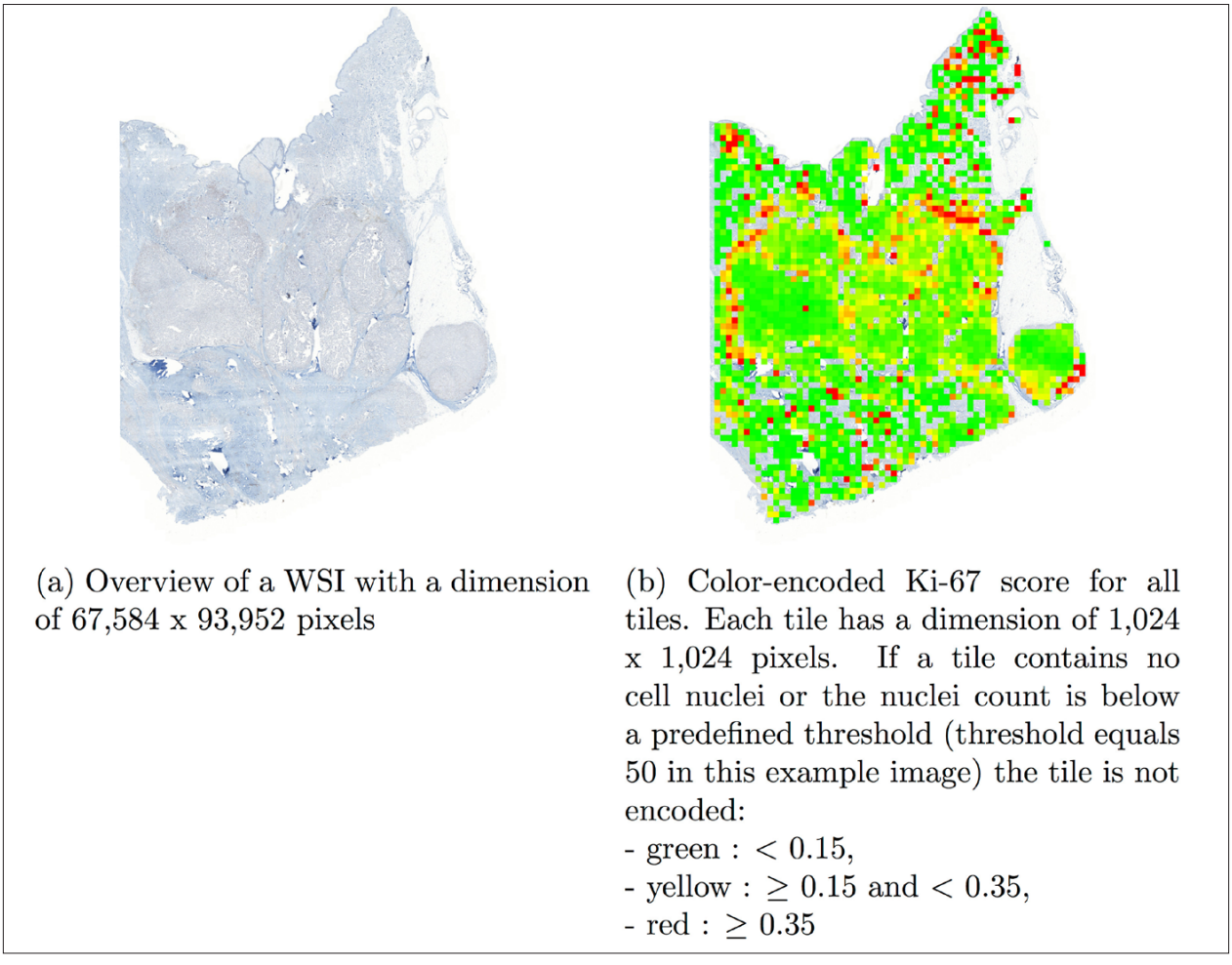
Over the last few years, the protein Ki-67 [1] has been established as one of the most important biomarkers for cell proliferation in breast cancer. High Ki-67 values indicate high tumor growth and have direct impact on the patient's treatment. Several automated image analysis methods for identifying Ki-67-positive and negative tumor cells have been presented.

Aims

For small regions of a virtual slide, the Ki-67 analysis can be realized within an acceptable period of time. However, to analyse an entire whole slide image (WSI [2]) most of the current methods are not sufficient yet. On a typical office computer, the processing time of 3,752 tiles, which were extracted from a H–DAB stained WSI, exceeded 24 hours. Therefore, we propose an approach to significantly speed up the process of analysing entire WSIs by using a distributed computing infrastructure.

Methods

To evaluate the approach, an unmodified and validated [3] [4] analysis software for Ki-67 was deployed on a six node setup supporting two different software engines: Hadoop Streaming [5] and Apache Spark [6] . Both tools support the MapReduce methodology whereas Apache



(a) Overview of a WSI with a dimension of 67,584 x 93,952 pixels

(b) Color-encoded Ki-67 score for all tiles. Each tile has a dimension of 1,024 x 1,024 pixels. If a tile contains no cell nuclei or the nuclei count is below a predefined threshold (threshold equals 50 in this example image) the tile is not encoded:
- green : < 0.15,
- yellow : ≥ 0.15 and < 0.35,
- red : ≥ 0.35

"Results of a Ki-67 analysis: (a) WSI overview image, (b) heat map of analysis result"

Spark offers alternative programing models. In addition, heat maps visualizing the Ki-67 scores for an entire slide were generated which can provide additional information for clinical research.

Results

First results on automated and reproducible tests have been produced. By processing 3,752 tiles the speedup turned out to increase linearly with the number of tiles. The overall processing time was improved by a factor of 10, more precisely from 28 hours on a typical office computer to three hours on a distributed environment. Further optimization strategies besides WSI partitioning will be considered. To achieve additional improvements in processing speed, the underlying algorithm of a Ki-67 analysis can be examined with focus on how to adapt it towards distributed processing workflows.

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COMPUTER-ASSISTED QUANTIFICATION OF CAIX MEMBRANE IMMUNOREACTION DESTINED FOR THE CLEAR CELLS IN RENAL CARCINOMA. A PILOT STUDY.

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Introduction/ Background

Carbonic Anhydrase IX [CAIX] has been considered as a candidate prognostic factor in clear-cell renal carcinoma [CCRC], however the supporting evidence is conflicting. CAIX is strongly induced by hypoxia via HIV-1α , and in CCRC via mutations to the VHL gene. CAIX expression could be identify as an immunohistochemical predictor of CCRC patients outcome but the published studies related to the patients prognosis have based on the diverse quantification protocols of CAIX expression (TMAs vs. whole tissue section; semiquantitative vs. computerised image analysis; with/without intensity scoring; with various software). The available commercial image analysis tools are mainly for general purpose e.g. software for breast carcinoma HER2 membrane immunoreaction has been used in various tumour tissue studies. However the cytological images of CCRC and breast carcinoma show essential differences related to the nuclei (size, outlines, intracellular location) and nuclear/cytoplasmic proportion which could influence the measurement credibility in maladjusted algorithm.

Aims

The aim of our study was to evaluate an algorithm for quantitation of the membranous CAIX expression specifically dedicated to CCRC ("snake variant") in comparative analysis to applied HER2 breast cancer algorithm for CCRC.

Methods

In the quantitative analysis of the specimen, the image processing follows: recognition of the cell nuclei; segmentation of the immunoreactive cell membranes; the assignment of the membrane segments to an individual cell. The last step is challenging for analysis due to frequent discontinuities in membranous immunoreaction, great variability of cellular counters and intracellular nuclei location. Because the classical watershed method for the individual cell separation is insufficient, the snake active contour method was applied, starting from each nucleus outline. The built gradient image allowed to select the most adequate parameters in the snake adaptation process. The recognized snake represents the membrane associated with the particular cell. The material includes records of 39 patients with the histopathologically verified diagnosis of CCRC who had nephrectomy (between 2009-2011) and were treated with tyrosine kinases agents (the Clinic of Oncology registry). 74% (29 out 39) of patients presented stage I - T1 N0; 20,5% - stage III and 5,4% stage TII. The formalin-fixed tissue sections of the resected CCRCs (the Pathology Department registry) were immunostained for CAIX protein using CAIX antibody (clone NB100-417) (Antibodies-online GmbH) with EnVisionTM (DAKO) according to the manufacture recommendations. The representative digital images were selected from each Whole Slide Image (scanned with Aperio, under 20x) and were assessed automatically by 3 independent observers using two algorithms: "snake variant" and "breast HER2". The extend of staining (percentage) was scored in the 10% intervals of CAIX positive carcinomatous cells and the intensity of immunoreaction was evaluated in 3 grade scale (1-3).

Results

The obtained results have been under investigation for the intra- and inter-observer accuracy as well as for the comparative data analysis of both types of algorithm. The statistical analysis has been incorporated. This approach explores a new possibility of the computerised quantitative estimation of the membrane CAIX immunoreaction destined for the specific clear cells in renal carcinoma.

CORRELATIONS BETWEEN INTERSTITIAL STROMAL FIBRILLARY NETWORK AND DISEASE PROGRESSION IN HEPATITIS C

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Introduction/ Background

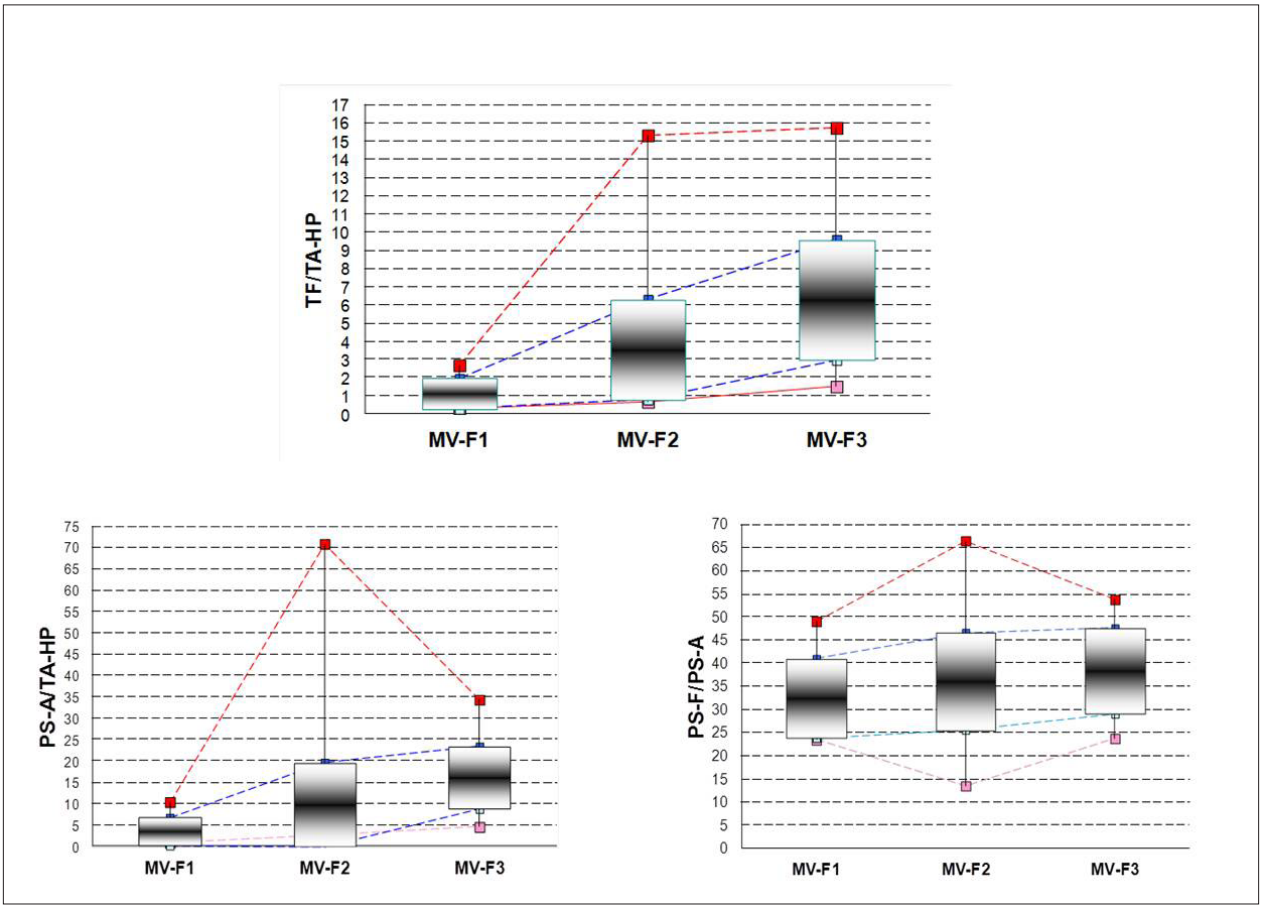
Since the virus description in the 80s [1] , [2], the infection with hepatitis virus C (HVC) became a real health problem because 50-80% of acute HVC infections evolve to chronic hepatitis, from which 4–20% of patients develop liver cirrhosis within 20 years and, finally, the risk of developing HCC of patients with liver cirrhosis is 1–5% per year [3]. The principal stage of pathological processes is the interstitial space and mainly the portal areas. Fibrillary network, one of the important components of the interstitial space, undergoes dramatic and highly variable changes, fibrosis representing one of the elements of the morphologic triad of the pathologic conflict within the liver parenchyma.

Aims

The aim of the study is to assess quantitatively the liver fibrosis on biopsy fragments of patients with virus C hepatitis (VCH) and to compare it with the fibrosis score described qualitatively in METAVIR system.

Methods

The studied material was represented by liver biopsies from 87 patients with VCH. Tissue fragments were processed following classical histological techniques (formalin fixation and paraffin embedment) and serial sections were stained, for each case, with Hematoxylin Eosin and Mason trichrome. Tissue fragments images were acquired with a dedicated optical system, using the



"Figure 1"

Computed Parameter	METAVIR Score	Statistical test – P-value (α=0.05)				
		t-test			ANOVA	χ2 test
TF/TA-HP	MV-F1	<0.00001		<	<0.00001	<0.0001
	MV-F2		0.0009			
	MV-F3			0.00001		
PS-A/TA-HP	MV-F1	0.0005		<	<0.00028	<0.0001
	MV-F2		0.0026			
	MV-F3			0.00001		
PS-F/PS-A	MV-F1	0,237		=	0,265	0,364
	MV-F2		0,352			
	MV-F3			0,079		

"Figure 2"

X10 objective and the portal and periportal areas were acquired using X20 objective. The fibrosis was firstly assessed using METAVIR qualitative score for fibrosis (MV-F1, MV-F2 and MV-F3). There were no cases with no fibrosis or with cirrhosis. The quantitative parameters determined were: total area of examined hepatic parenchyma, portal spaces area, total area of fibrosis and area of portal fibrosis. The quantitative parameters calculated were: the percentage of the total parenchymal area represented by fibrosis (TF/TA-HP), the percentage of the parenchymal area represented by the portal spaces (PS-A/TA-HP), the percentage of the portal spaces represented by the portal fibrosis (PS-F/PS-A). The measurements were made with two dedicated software programs, after preceding software calibration. For numerical parameters minimum (MIN), maximum (MAX) mean (AV) values and standard deviation (STDEV) were calculated. For comparison with METAVIR fibrosis score grades, the values of quantitative parameters calculated were stratified in classes. For statistical analysis of the correlation between the quantitative and qualitative assessment of fibrosis, t-test (2-sample, unequal variance), One-Way ANOVA test and χ2 test were used.

Results

TF/TA-HP and PS-A/TA-HP correlated with the METAVIR degrees of fibrosis *(Figure 1)*. Both correlations were statistically validated at very high significance level. *(Figure 2)*. In turn, PS-F/PS-A didn't correlate with the METAVIR degrees of fibrosis, as statistical tests revealed *(Figures 1 and 2)*.

So, in VCH, one of the main morphological aspects is the constant enlargement of portal spaces but with a reduced extension of the destructive and reparatory processes towards the lobule center. Collagen fibers production is not an accelerated process, being in a relative equilibrium with the reactive inflammatory cellular population as demonstrated by the relatively constant percentage of the portal spaces represented by the fibrillary structures

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DETECTION OF AUTOMATIC DIGITAL IMAGE ANALYSIS PROBLEMS FOR THE EVALUATION OF IMMUNE MARKERS IN BREAST CANCER BIOPSIES

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Introduction/ Background

Automatic digital image analysis has increased in recent years. There are several applications for image analysis in pathology, among them, immunohistochemistry biomarkers quantification. This is a simple, economic and fast method to quantify stained biomarkers in digitalized biopsies enhancing sensitivity and objectivity. However, automatic procedures do not always work satisfactorily in all digitalized samples.

Aims

To quantify the number of incorrectly analyzed images as a result of errors on the automatic procedure developed for quantifying immune markers in breast cancer biopsies, to evaluate the amount of time spent in this reanalysis and to define which kind of biopsy produces more errors.

Methods

10,770 cores of breast tumor (intratumoral and peritumoral areas) and negative and positive axillary lymph nodes areas from ductal invasive breast cancer were included in different tissue microarrays (TMAs). Slides of each TMA were immunohistochemically stained for CD4, CD8, CD57, CD68, S100, LAMP3, CD83, CD1A, CD123 and CD21 immune markers and were digitalized at 40X with the Apperio Scanscope XT scanner. Each core was extracted as an individual digital image in TIFF format. The stained area was automatically quantified using procedures developed with Fiji (Image J) software in a HP Intel Inside Core i.7 computer with 16GB of RAM memory. Firstly, the whole area of each core was evaluated in pixels by using the luminance channel, applying the median filter and gray-scale segmentation. The second step evaluated the positive pixel number stained in brown for obtaining a brown color channel and then, a gray scale and size segmentation for positive objects, including holes inside the segmented area. Finally, this selected brown area was automatically surrounded by an overlay.

Results

2,751 had to be reanalyzed (25.5%). Specifically, intratumoral and peritumoral cores were those with higher reanalysis levels (35.6% and 34.9%, respectively) while axillary lymph nodes cores present lower levels (Negative Nodes 13.5% and Positive Nodes 16.4%). Regarding the immune biomarker, CD21, LAMP3 and CD123 were those with higher reanalysis levels (38.4%, 35.2% and 34.2%, respectively) in contrast with CD8, CD68 and CD83 that were those with lower levels (16.4%, 17.7%, 18.4%, respectively). The reanalysis levels of the remaining biomarkers were 22% in CD4, 23.9% in S100, 26.% in CD1A and 27.4% in CD57. Each core took a mean of 1.56 minutes to be analyzed so the total time spent in the reanalysis of the images was 71.5 hours. The principal reasons for reanalysis were problems in the TMAs assembly accuracy and presence of adipose tissue, hemosiderin, artifacts, unspecific staining and background noise. Several TMAs presented glue bubbles and different types of dirt, as hairs or dust that were quantified as positive area. Intratumoral and peritumoral cores are those with higher levels of adipose tissue. These adipocytes cause alterations in the automatic quantification due to their different cellular structure. Specifically, in cores stained for S100 marker in which membranes of adipocytes were also stained in brown and, thus, quantified as immune marker.

Conclusions: It could be possible to reduce the time of analysis and to obtain more exact values of immune quantification in digital images improving the accuracy of TMAs assembly, overcoming unspecific staining and background and adapting the parameters of the procedures.

SEMI-AUTOMATIC AND AUTOMATIC KI-67 INDEX EXAMINATION IN WHOLE SLIDE IMAGES OF MENINGIOMAS

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Introduction/ Background

Histological examination of tissue subjects by immunohistochemical staining is the basic method of recognizing types of cancer and it provides valuable indicators concerning choice of optimal therapy or defining the prognosis. One of a most important markers is the mitotic receptor Ki-67, among others, in maningiomas [1]. According to examination guidelines, ROI's (Region of interest) whose fields correspond with the high positive receptors' reaction should be selected.

Aims

The aim of this paper is a compare of Ki-67 index examination in meningioma specimens performed on the whole slide images(WSI) in two ways: with selection of hot-spot regions by the experts, and with automatic selection of hot-spots. Using both ways we have analyzed variability of results between two experts and between the exprets and the automatic procedure, also in respect of Ki-67 level.

Methods

The fifty cases of meningiomas were stained with the ready-to-use FLEX Ki-67 antigen (Dako, code IR626) in Dako Autostainer Link. Acquisition of WSIs was carried out by the 3DHistech Pannoramic 250 Flash II scanner under the 20x magnification of lens. The selection of hot-spots was done manually by two experts and automatically with the proposed method of automatic hot-spot detection. The suggested WSI processing scheme was based on the following steps:

- defining the map of specimen using the thresholding procedure and morphological filtering,
- eliminating the areas containing blood cells (hemorrhages) by the texture analysis (Unser features) and classification,
- eliminating the specinem folds by the texture analysis (Unser and Local Binary Patterns) and classification,
- selecting sequential fields of the hot-spots based on cells segmentation and the punishment function to avoid excessive proximity, and it is the extention of idea presented in paper [2]. The final analysis of Ki-67 index was performed on the full resolution images with the same procedure of image analysis.

Results

The results indicated that the mean difference between the Ki-67 index of Expert A and Expert B was -0.6065% (SD ±1.27%). Comparison between the results of Automatic system and Expert A gives mean difference 0.5207% (SD 1.18%) whereas in relation to the Exert B, it was -0.0858% (SD 1.21%). No significant skewness was observed in any of Bland-Altman plots.

The determination analysis gives R^2 equals 0.947 (Expert A to Expert B), 0.947 (System to Expert A), and 0.944 (System to Expert B), all $p<0.000001$. The automatic procedure for the hot-spot detection in meningioma WSI gives the high concordance of results with the expert's examinations. The differences between the automatic and both experts' results are included in the range of variability of experts' results. The presented results confirm that the proposed automatic procedure can be introduced to the multicenter verification process for practical applicability in histopathological diagnosis in the near future. This work has been supported by the National Centre for Research and Development (PBS2/A9/21/2013 grant), Poland.

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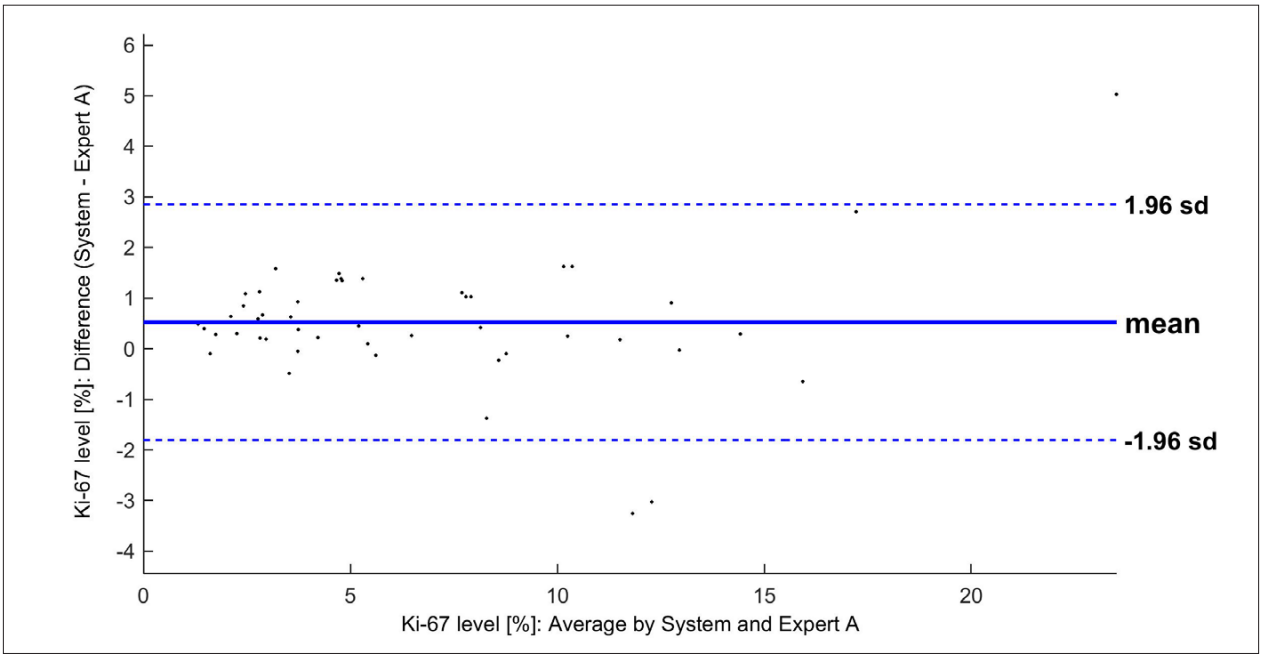

"Bland-Altman plot for System to Expert A Ki-67 evaluation."

OBJECTIVE KI-67 INDEX QUANTIFICATION IN NON-BREAST TUMORS – PRELIMINARY DATA IN TIMISOARA, ROMANIA

A. Vaduva*[1], R. Cornea[1, 2], I. Mihai[1, 2], M. Cornianu[1, 2], D. Szilagyi[2], A. Muresan[1, 2], D. Anderco[2], C. Suciu[2], C. Lazureanu[1, 2], A. Dema[1, 2]

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Introduction/ Background

The immunohistochemical expression of the Ki-67 protein is routinely used for diagnostic and grading purposes of various tumors. The most common use of automation process for Ki-67 quantification is represented by breast tumors.

Aims

We aimed to analyze the accuracy of digital quantification of Ki-67 expression in non-breast tumors.

Methods

We selected all the available Ki-67 stained slides done in 2014 at the County Emergency Hospital Timisoara, Romania, excluding breast tumors. Images of the stained slides (10 images/slide on average, depending on the size of the specimen) were acquired on a Leica DMD108. We performed preprocessing (autolevels) in IrfanView and Ki-67 quantification in Fiji (ImageJ distribution) using ImmunoRatio plugin.

Results

Proper image segmentation was identified on 25 cases. The best segmentation was obtained in lymphoid and central nervous system (CNS) tumors. Lower Ki-67 values than the ones manually reported were identified in 16/25 cases (64%). Interestingly, digital quantification showed all 7 lymphoid cases to have a lower Ki-67 index than manually reported, while 6/9 CNS had higher Ki-67 indices using automation processing. Valid digital quantification was not possible on skin, cervical and urothelial tumors, as regions of interest could not be defined to restrain the area to be evaluated.

Conclusion: Our preliminary study shows that ImmunoRatio plugin for Fiji in combination with IrfanView preprocessing are free, easy to use and powerful tools to obtain an objective Ki-67 index in non-mammary tumors: CNS, lymphoid, soft tissue, neuroendocrine tumors, choriocarcinomas and malignant melanomas.

PATCH-BASED NONLINEAR IMAGE REGISTRATION FOR GIGAPIXEL WHOLE SLIDE IMAGES

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Introduction/ Background

Image Registration of whole slide histology images allows the fusion of fine-grained information - like different immunohistochemical stains - from neighboring tissue slides. Traditionally, pathologists fuse this information by looking subsequently at one slide at a time. If the slides are digitized and accurately aligned at cell-level, automatic analysis can be used to ease the pathologist's work. However, the size of those images exceeds the memory capacity of regular computers, preventing the application of established image registration methods at a high magnification.

Aims

An accurate and automatic alignment helps the pathologist to analyze the combination of different antibodies without memorizing multiple slides. For some applications, cell-level accuracy is needed. This also enables automatic image analysis to take advantage of multi-slide information.

Methods

We extend available registration methods by using image data at fine spatial resolution. However, this data is not simultaneously globally available due to the computer's memory restrictions.

Typical approaches either reduce the amount of data to be processed by downsampling or partition the data into smaller chunks to be processed separately. We combine the patch based approach with an elastic deformation model to obtain a global registration result.

Our method first registers the complete images on a low resolution with a nonlinear deformation model and later refines this result on patches by using a second nonlinear registration on each patch. The deformation information on the individual patches can be contradictory and needs to be combined into one global model. As an extension to our previous work, the global solution is computed by minimizing an energy function that preserves the patch-wise deformation and establishes global smoothness.

The NGF distance measure is used to handle multi-stain images.

Results

The method will be applied to whole slide image pairs. The alignment of corresponding structures will be measured by comparing manual segmentations from neighboring slides. First results show an improvement of the registration accuracy compared to the low-resolution nonlinear registration.

KN02.01 | **Keynote II - Liron Pantanowitz**

STRATEGIES AND DEMANDS FOR DIGITAL PATHOLOGY WORKFLOW INTEGRATION

L. Pantanowitz*

University of Pittsburgh, Medical Center, Pittsburgh, United States

Digital Pathology has many benefits and pathology laboratories around the world are capitalizing on many of these applications including education, telepathology, and image analysis. However, if not implemented well digital pathology can have both positive and negative impacts on workflow. The key is to ensure that the digital imaging solution selected overall enhances workflow. Batched scanning, failed scans and downtime are examples where whole slide imaging can negatively impact workflow. High speed digitization, load balancing, and smart algorithms on the other hand can all improve workflow. Optimal image management and integration with the laboratory information system are also essential for sustaining an efficient digital pathology workflow. The aim of this talk is to address many of these critical factors, their impact on digital pathology workflow, and to discuss novel opportunities that by enhancing workflow will help evolve the practice of pathology.

USE OF DIGITAL IMAGE ANALYSIS FOR OUTCOME PREDICTION IN BREAST CANCER

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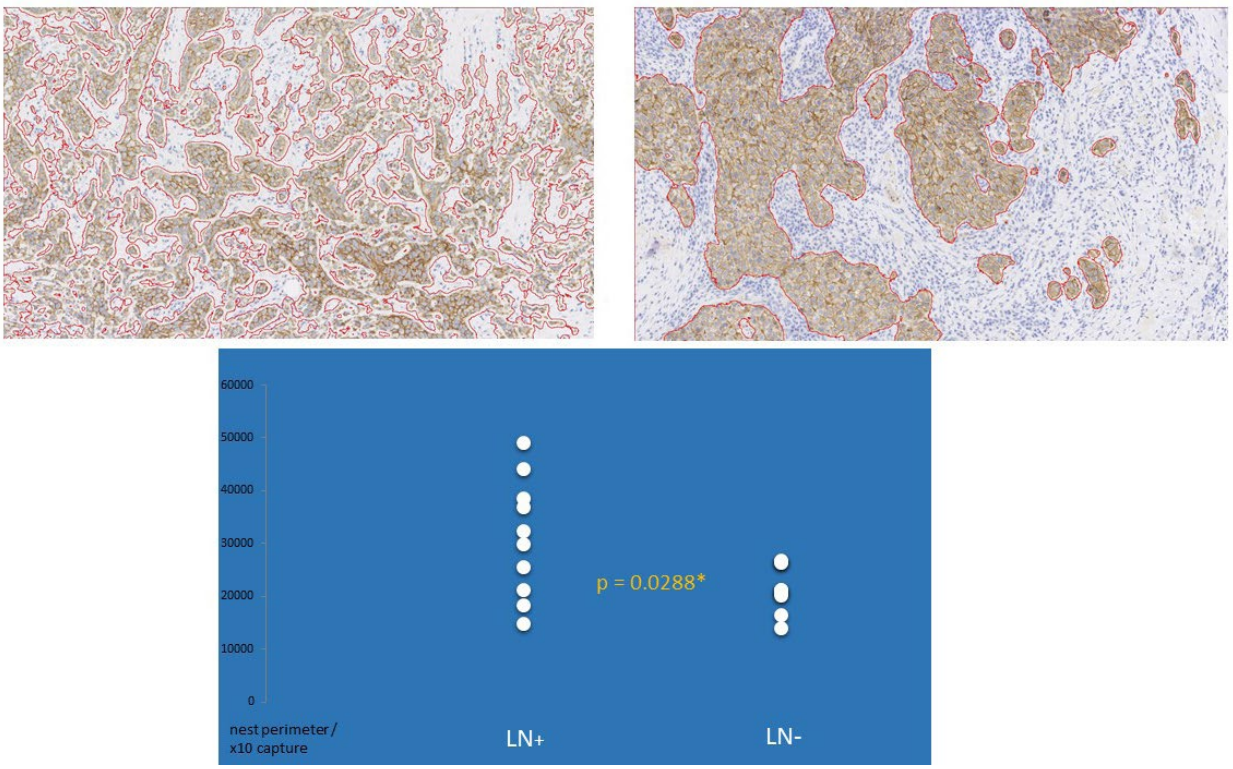
Introduction/ Background

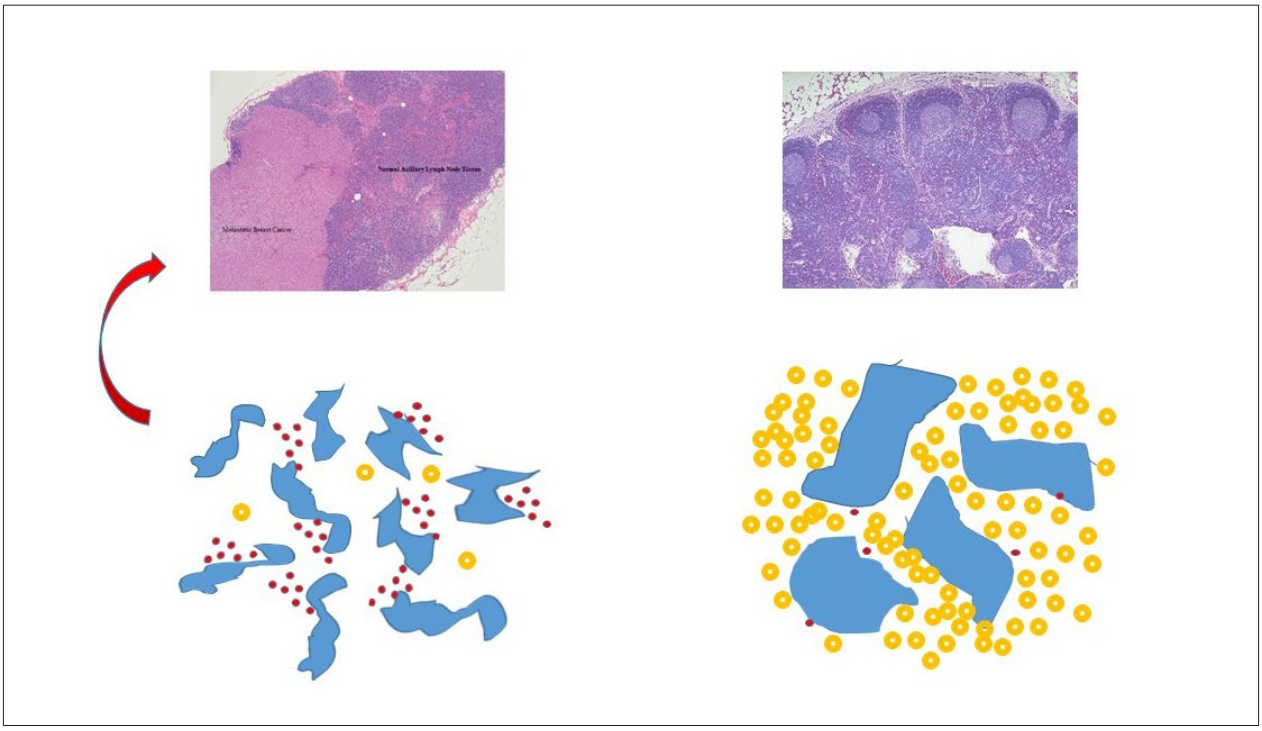
Breast cancer is the most common cancer in the UK. Although 10 year survival has increased over last decades, significant improvement is still needed. Clinical management decisions are largely dependent on assessment of histological features. The traditional approach to histopathological assessment has been the expert manual reporting of cases as viewed with a light microscope and has remained virtually unchanged since 1928, with minor modifications that have led to the current routinely applied semi-quantitative tumour grading system. However, the abundance of information within the tumour microenvironment is not reflected in the traditionally evaluated histological features, and there remain morphological features with prognostic potential that have previously been beyond investigation by traditional manual microscopic means. Tumour prognosis is closely related to metastasis, a complex process involving tumour cell migration through the stromal microenvironment before entering the lymphovascular compartment. Tumour/stromal interaction is crucial in the process and represents a potential candidate for therapeutic intervention. This interaction is partly affected by the pattern of tumour migration, revealed in the tumour architecture, and partly by the stromal response.

Aims

Our working hypothesis for the proposed study framework was that, with the application of digital image analysis technology, previously unquantifiable tumour architectural and microenvironmental features can be



"Total tumour nest perimeter and lymph node status"



"Tumour architectural and stromal features associated with lymphatic spread"

rigorously assessed in detail and tested as potential prognostic parameters. Quantified features included tumour extracellular particles at the tumour-stroma interface, tumour infiltrating lymphocytes, tumour nest perimeter, number, size and shape. The selected prognostic parameter was axillary lymph node metastasis.

Methods

Our initial study included diagnostic core biopsies from 19 HER2 positive breast cancers, with approximately equal number of ER strongly positive or weakly positive/negative cases. Her2 immunohistochemistry allowed rigorous segregation of epithelial elements. Immunostained sections were digitised using a Hamamatsu scanner and x10 magnification consecutive segments from .ndpi files were captured as .jpeg files and analysed using Fiji (Image J), a public domain image processing program. The entirety of each core was examined in all cases. Several native Fiji Functions and Fiji plugins, including Trainable Weka Segmentation, Colour Segmentation and Colour Deconvolution were employed in different combinations for different types of analysis. The analysis is currently being expanded to a large set of digitised breast cancer tissue microarray (TMA) slides which have been stained with cytokeratin to highlight tumour cells. The set includes breast cancer cases from all molecular subtypes and is linked with detailed histological and outcome data.

Results

Increased number of extracellular particles at the tumour-stroma interface and decreased number of tumour-infiltrating lymphocytes were significantly associated with axillary lymph node metastasis (p=0.0062 and p=0.0154 respectively). Combination of the two parameters increased further the strength of the association (p=0.0011). Increased total tumour nest perimeter, tumour nest number and tumour nest shape irregularity were also significantly associated with axillary lymph node metastasis (p=0.0288, p=0.0085 and 0.0203 respectively).

Data from the analysis of TMAs are currently analysed and will be presented

IMAGE ANALYSIS APPROACH TO DISTINGUISH LOBULAR STRUCTURES IN THE MAMMARY GLAND FROM WELL-DIFFERENTIATED BREAST CANCER WITH TUBULE FORMATION

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Introduction/ Background

Automated detection of diagnostically relevant regions of interest (ROI) is one of the major challenges in medical image analysis. In digital pathology using whole slide images (WSI), the detection of certain biological structures is necessary, because the capacity for analysis of cells and subcellular structures at high resolution is limited by available processing time and computational power. For some applications it is also important to exclude irrelevant or misleading components of the image. One important challenge of this type is well differentiated (low-grade/G1) breast cancer.

Aims

We aim at automatically distinguishing non-malignant lobular tissue from well-differentiated breast cancer to avoid erroneous evaluation of normal tissue in the detection of nuclear hormone receptor expression. A second goal of lobule detection is to exclude inflammation of non-malignant pre-existing structures from tumor immune cell scoring of breast cancer in oncoimmunology.

Methods

Two approaches for lobule detection were applied: The first includes modules of own image analysis algorithms developed specifically for lobule detection combined with elements of a commercially available software platform (Definiens Developer®), and the second is a software package optimized for use with a multispectral camera system (Inform, Perkin-Elmer ®). Breast cancer samples were stained for estrogen receptor (ER), progesterone receptor (PR) and the lymphocyte marker CD8. The first approach starts with a texture-based supervised classification to detect lobule candidate regions and uses a nuclear density image to refine the candidate regions. The second approach uses a supervised machine learning method whose features and algorithm are not disclosed to the user. Manual annotations of lobular tissue by expert pathologists were used for evaluation of results.

Results

The accuracy of distinction between cancer areas and lobular structures decreased in cases with prominent glandular differentiation of the tumor. The major challenge was the separation of well-differentiated (G1) breast cancer with consistent hormone receptor expression from adjacent lobular areas. The second approach performed well on high-grade tumors and had advantages regarding speed and its convenient user interface. The modular first approach was superior on ER and PR images and successfully detected lobular areas even if the anatomical structure was disrupted by inflammation of infiltrating cancer cells. We conclude that modular approaches considering image context and allowing specific adjustments to the tissue of interest may be needed to overcome current limitations of automated ROI detection in clinical biopsy materials.

AUTOMATED KI67 HOTSPOT DETECTION FOR BREAST CANCER BIOPSIES

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Introduction/ Background

The quantification of immunohistochemical Ki67 and the detection of active areas of tumor cell proliferation (hotspots) have a critical importance in the prognosis and treatment planning for breast cancer.

Aims

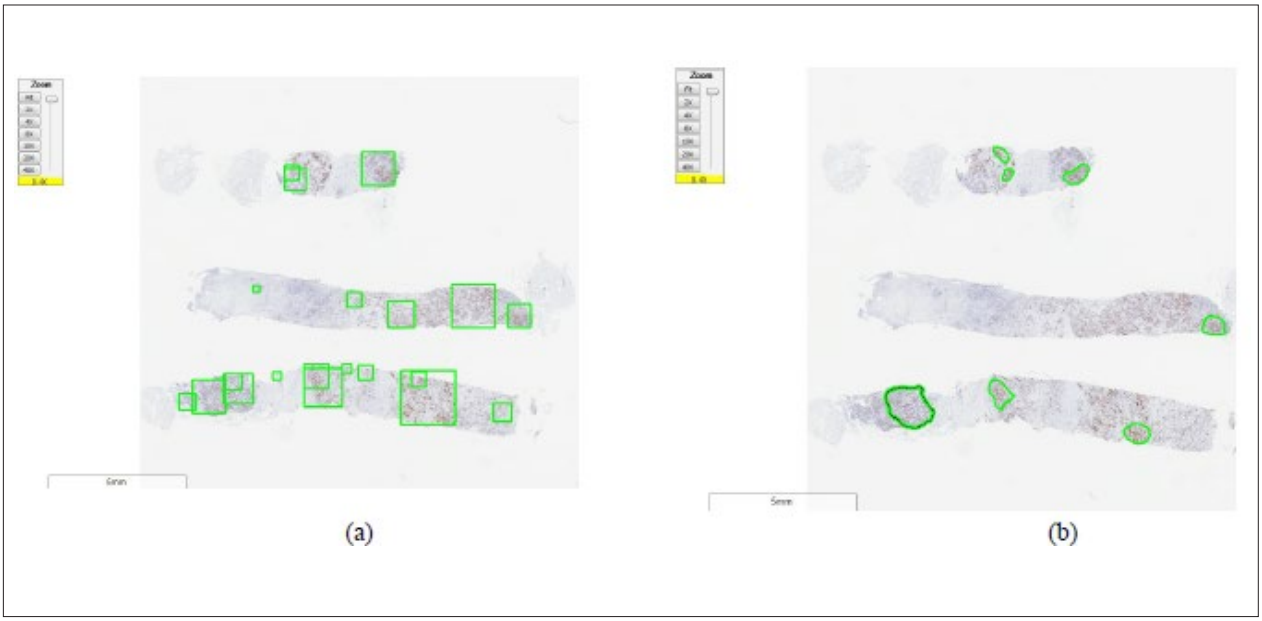
In this work an automated and robust method for the detection of hotspot areas in breast cancer biopsies is proposed with the aim of supporting the pathologists by highlighting hotspot areas.

Methods

The proposed method has been tested on one Ki67 stained image from Openslide [1] acquired at 40x with an Hamamatsu scanner and on 5 Ki67 stained images of breast cancer biopsies acquired at 40x with AperioCS. Each input image is divided in tiles, whose colors are deconvolved using the method of Ruifrok [2] to estimate the presence of Ki67. Tiles with an estimated Diaminobenzidine (DAB) positivity of more than 5% are considered as potential hotspots. Neighbouring positive tiles are merged to form a final hotspot area. The three hotspot areas with higher DAB positivity are also highlighted in the output. The hotspot areas are then written in an XML file which is read by a medical image viewer such as Aperio ImageScope. The proposed method has been implemented in Java using the BioFormats opensource library [3].

Results

The color deconvolution of each tile has been performed by applying the standard Hematoxylin/Diaminobenzidine (H/DAB) deconvolution matrix provided in Fiji [4] . The tests have been performed at different zoom levels resulting in similar, coherent outputs of hotspot areas. The resulting hotspot areas have been validated by visual comparison with the hotspot areas determined by experts, showing a significant superimposition as shown in Fig. 1. The proposed method has been compared with the ASH method [5] for the image acquired with Hamamatsu scanner, producing similar results in comparable time.



"Fig. 1: In (a) are highlighted the hotspot areas resulting from the proposed method over a H/DAB stained image of breast cancer biopsy. They significantly match with the hotspot areas determined by expert pathologists in (b)."

In ***conclusion***, a new fully automated method for the detection of Ki67 hotspot areas in breast cancer biopsies has been proposed to support the pathologist by highlighting different hotspot areas. It is able to process different medical images formats, making it more interoperable. The time performance is comparable with existing methods such as ASH [5] . The proposed method at low magnification such as 1x, 2x, and 4x produced varying results influenced by the size that each tile assumes at such magnification levels. Further extension of the proposed method will also include the MIB-1 estimation within the hotspot areas as well as extensive testing and validation. This work is partially founded by the EU FP7 program, grant number 612471.

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AUTOMATIC DETECTION OF SUSPICIOUS REGIONS IN WHOLE SLIDE IMAGING FOR PATIENTS WITH BARRETT'S ESOPHAGUS

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Introduction/ Background

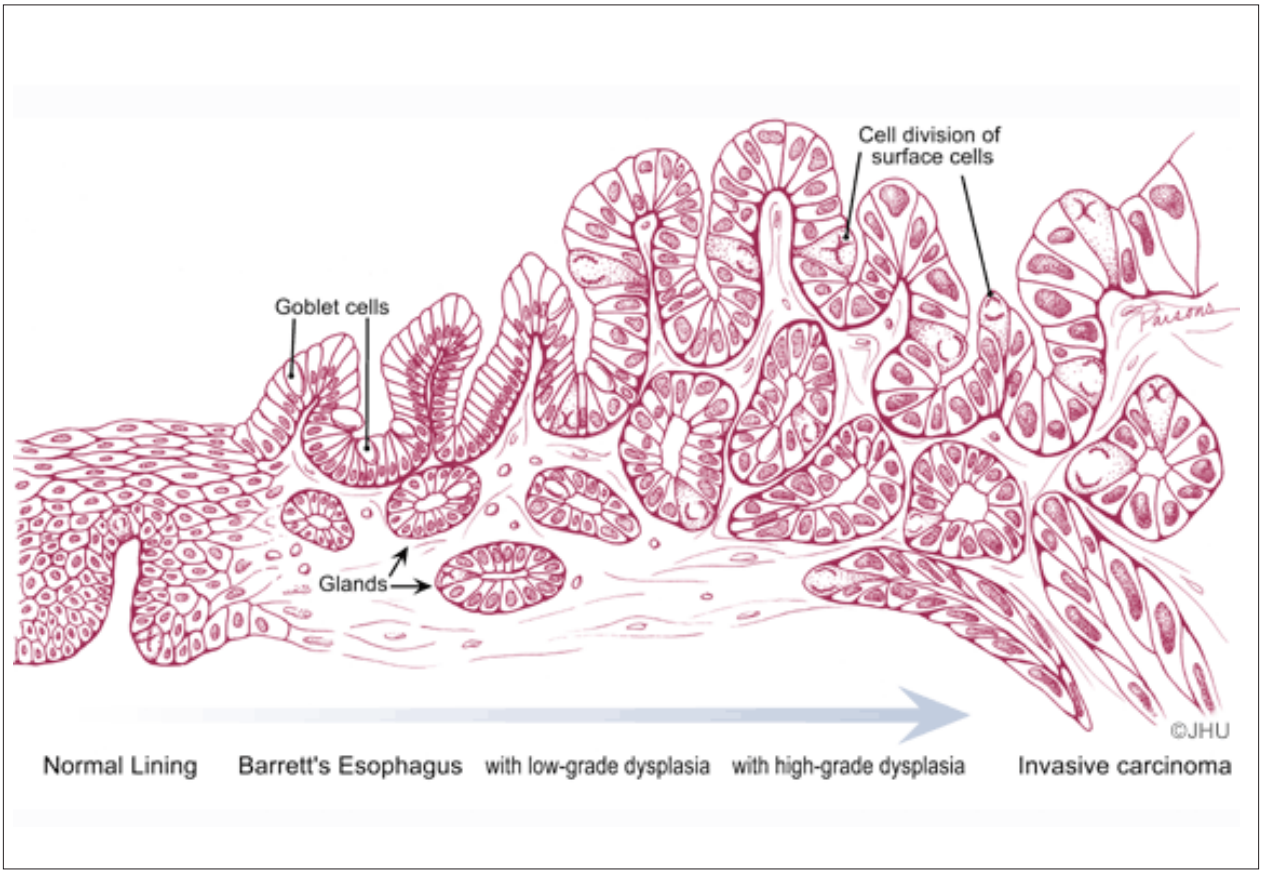
Barrett's esophagus is a premalignant condition, in which normal stratified squamous epithelium in the lower portion of the esophagus has been replaced by simple columnar epithelium with goblet cells. Barrett's esophagus has a strong association with esophageal adenocarcinoma, notorious for a high mortality rate as well as a large impact on quality of life. Differentiation between dysplasia and adenocarcinoma on histology slides is time-consuming and subject to inter- and intra-observer variation. For this reason, automatic detection of suspicious regions in esophageal biopsies would advances accurate diagnosis and could contribute to reduce the workload for pathologists.

Aims

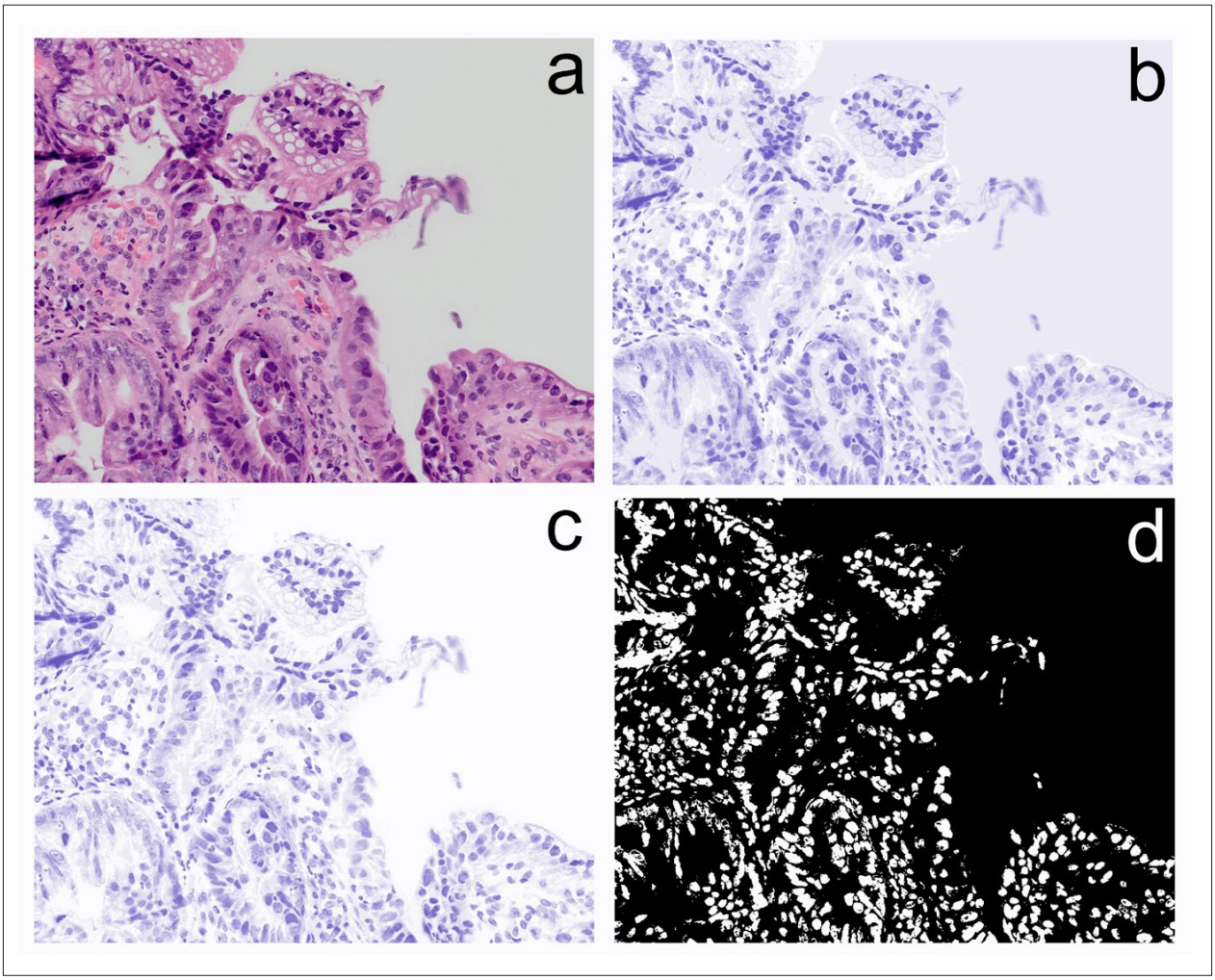
The objective of this study is to accurately detect suspected regions using pixel-level and object-level features.

Methods

A dataset of twenty patients with Barrett's esophagus was acquired, containing two microscopic images from endoscopic resection specimens per patient. The biopsies were formalin-fixed paraffin-embedded (FFPE), processed and hematoxylin-eosin (H&E) stained. The slides were scanned using an Olympus dotSlide scanner with a 20x objective (Fig 2a). Color deconvolution was performed to separate the hematoxylin (nuclei) and



"Fig 1: Simplified representation of the different stages in Barrett's esophagus progression (http://pathology2.jhu.edu/)."



"Fig 2: Processing of a Barrett's esophagus with high-grade dysplasia."

eosin (cytoplasm) signal (Fig 2b). Subsequently, background subtraction (Fig 2c) was performed followed by segmentation of the nuclei (Fig 2d). Semantic-level features, e.g. density and area of the nuclei, eccentricity and clustering of nuclei were extracted as well as texture and topology of the tissue. These features were compared with the regions of interest of two individual expert pathologists.

Whole slide imaging (WSI) images are subdivided in small tiles of approximately 1000x1000 pixels. Tiles with less than 5% tissue will be disregarded immediately. Based on the semantic features, texture and topology of the tissue, each tile is classified as suspicious or not suspicious.

Results

An example of the first steps in image processing of these specimens can be seen in Figure 2, showing the potential value of this automated approach.

AUTOMATED MEASUREMENT OF THE DENSITY OF VESSELS ON WHOLE SLIDE IMAGES OF PAEDIATRIC BRAIN TUMOURS

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Introduction/ Background

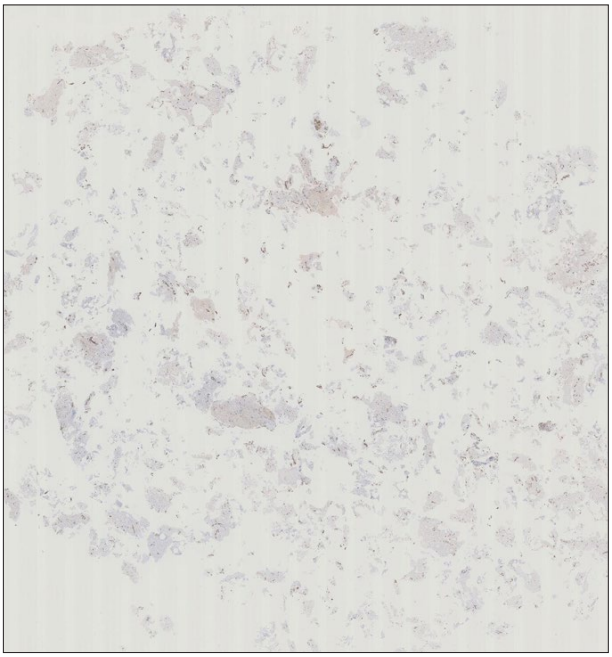
Microvasculature is known to have a prognostic significance in many brain tumours. It is important to be able to quantify it in a reproducible way on biopsy samples and, if possible, through noninvasive imaging techniques.

Aims

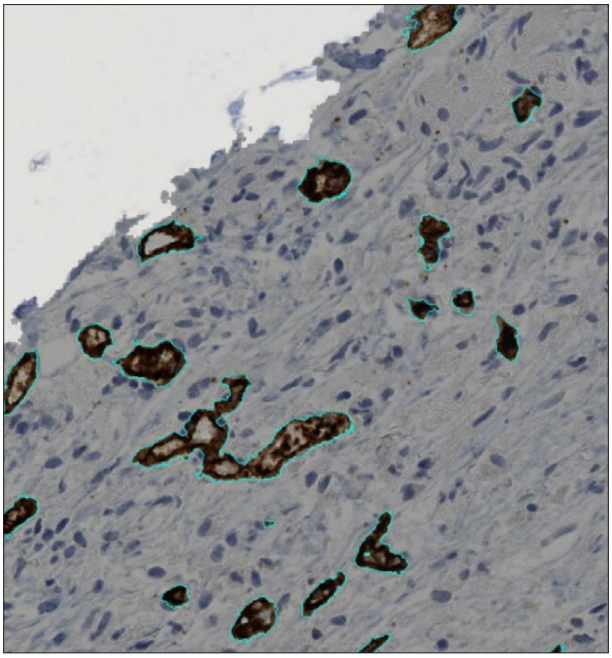
A quantitative and reproducible way to assess the microvasculature of biopsy samples of human paediatric brain tumours is needed, in order to help diagnosis (possibly give a hint of the grading of the tumours) and to see if a correlation exists with a perfusion MRI (ASL) signal. The method should be applicable to standard whole-slide images of samples immunostained with CD34 marker over haemalum. Such slides are usually very fragmented, which makes the manual measurement of density particularly tedious and error-prone.

Methods

Our method starts with a precise determination of the zones of tissue on the whole-slide image. The we measure their area and we calibrate automatically the staining optical densities and the background colour. Automated detection, measurement and counting of vessels takes place, based on a colour deconvolution [1] followed by automatic thresholding [4] and finally morphological operations to remove artefacts (e.g., dust). In order to deal with the large size of whole-slide images, many of which exceed the capacity of the computer's memory, we proceed on rectangular extracts of the whole image. The whole treatment was performed on a standard desktop computer with 16 GiB of RAM [2]. In the end, masks of areas considered as tissue and as vessel walls and lumina are produced and uploaded, along with the virtual slide, to a webserver



"Example of digitized slide from our series of samples of paediatric brain tumours. The image size is 99200x98560 pixels (real size 2.24x2.23cm). Notice that tissue is highly fragmented."



"Example of segmentation of vessels on one of our samples, as can be seen in a web browser for quality control by the pathologist. Area considered as tissue without blur is shaded. Areas considered as vessels are contoured in cyan."

This allows the pathologist to check the quality of segmentation from his office using a simple JavaScript-capable web browser. We tried two formats for web serving: DeepZoom (which can be served directly by a webserver) and pyramidal TIFF (which needs to be served by a modified version of IIPImage server [3] because lossless compression of the masks was used to preserve both disk space and image quality).

Results

Only a few parameters have to be chosen, once and for all samples (e.g., the minimal acceptable size of a blood vessel fragment), which makes the method more robust than assessment by a (panel of) human expert(s). The automatic calibration steps enable one to deal with a heterogeneous set of slides (e.g., slight differences in background colour and staining). The method uses only open-source software. It is easy to extend or improve and not tight to a single immunomarker.

We applied the method to 129 paediatric brain tumours of 8 different types and 3 locations (posterior fossa, thalamus, hemispheres) — 185 samples in total. For each patient, the density of microvessels in the sample is compared to the cerebral blood flow as assessed by preoperative perfusion-weighted-imaging using arterial-spin-labeling. We find a good correlation between microvascular density, MRI data and tumour grading. The microvascular density is broadly distributed among the samples

Visualisation in a web browser is slightly more fluid when images are uploaded in the DeepZoom format rather than as pyramidal TIFF images, but the former consumes roughly 20 times more disk space and needs the transfer of a very large number of files after each modification, which is less tractable.

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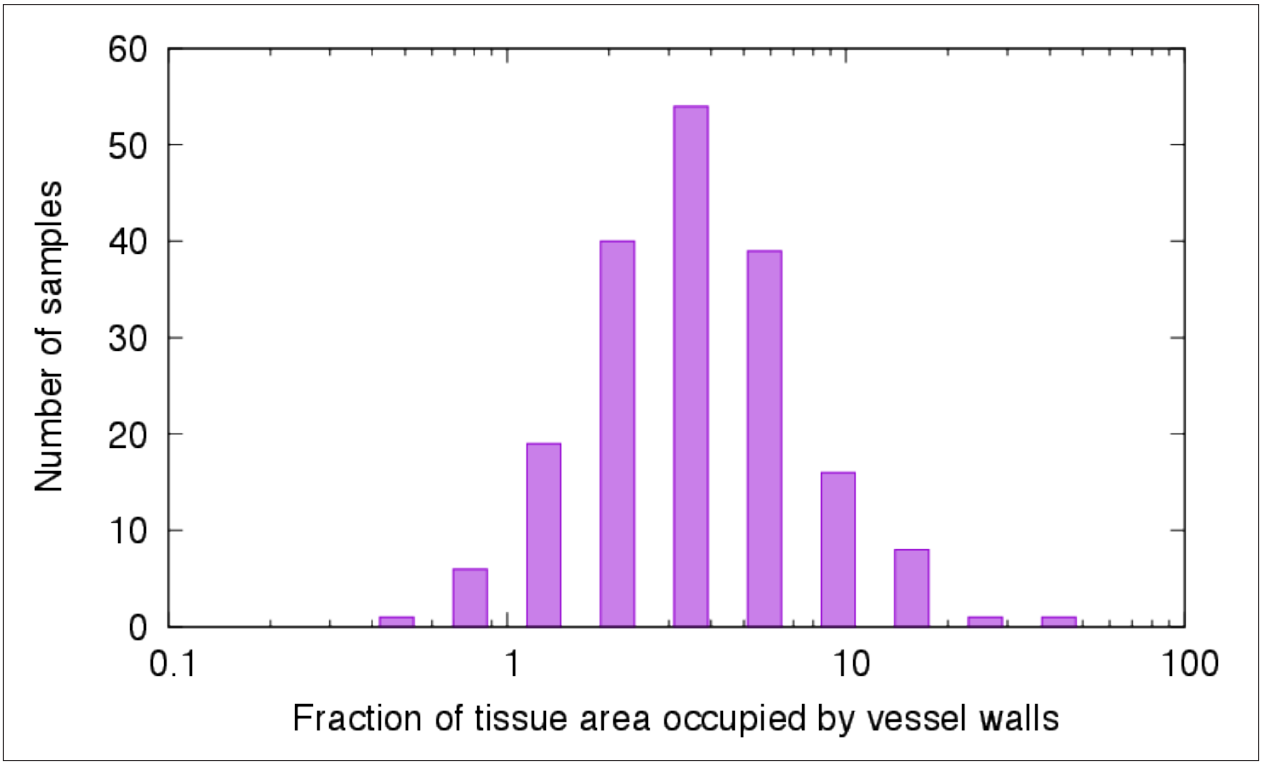

"Distribution of the fraction of tissue area occupied by the vessel walls in our set of 185 samples."

MICROMETASTASIS DETECTION GUIDANCE BY WHOLE-SLIDE IMAGE TEXTURE ANALYSIS IN COLORECTAL LYMPH NODES

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Introduction/ Background

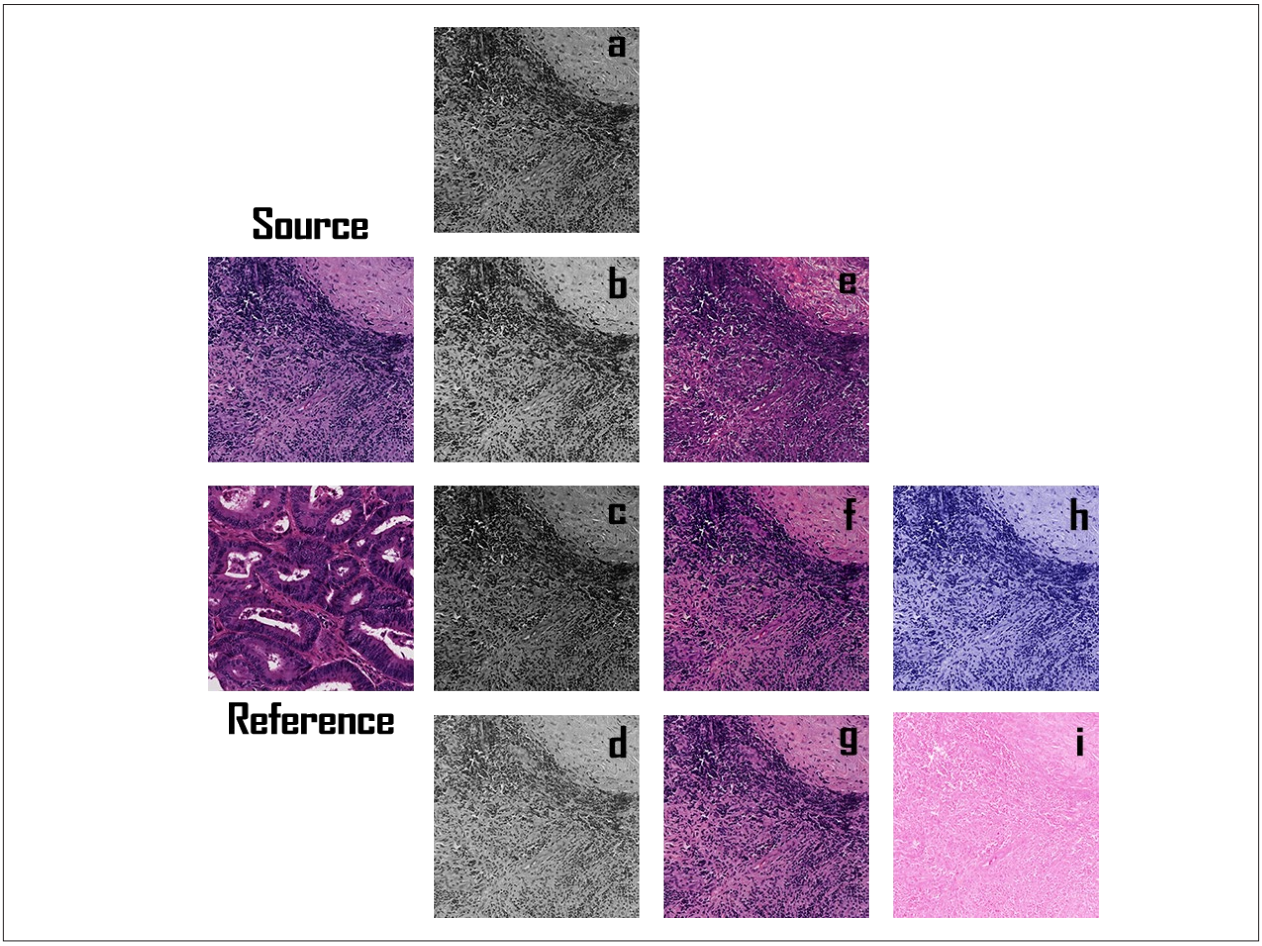
Cancer is a disease that affects millions worldwide and accurate determination of whether lymph nodes (LNs) near the primary tumor contain metastatic foci is of critical importance for proper patient management. Histopathological evaluation is the only accepted method to make that determination. However, the current standard of care only examines a single central histological section per LN and yields an unacceptable false-negative rate.

Aims

To help pathologists in their examination we propose a method that extracts textural features from histopathological LN whole slide images (WSI) and then applies support vector machines (SVMs) to automatically identify regions suspicious for metastatic foci.

Methods

The database consisted of WSI from 44 LNs. Sections were stained with hematoxylin-eosin and examined at

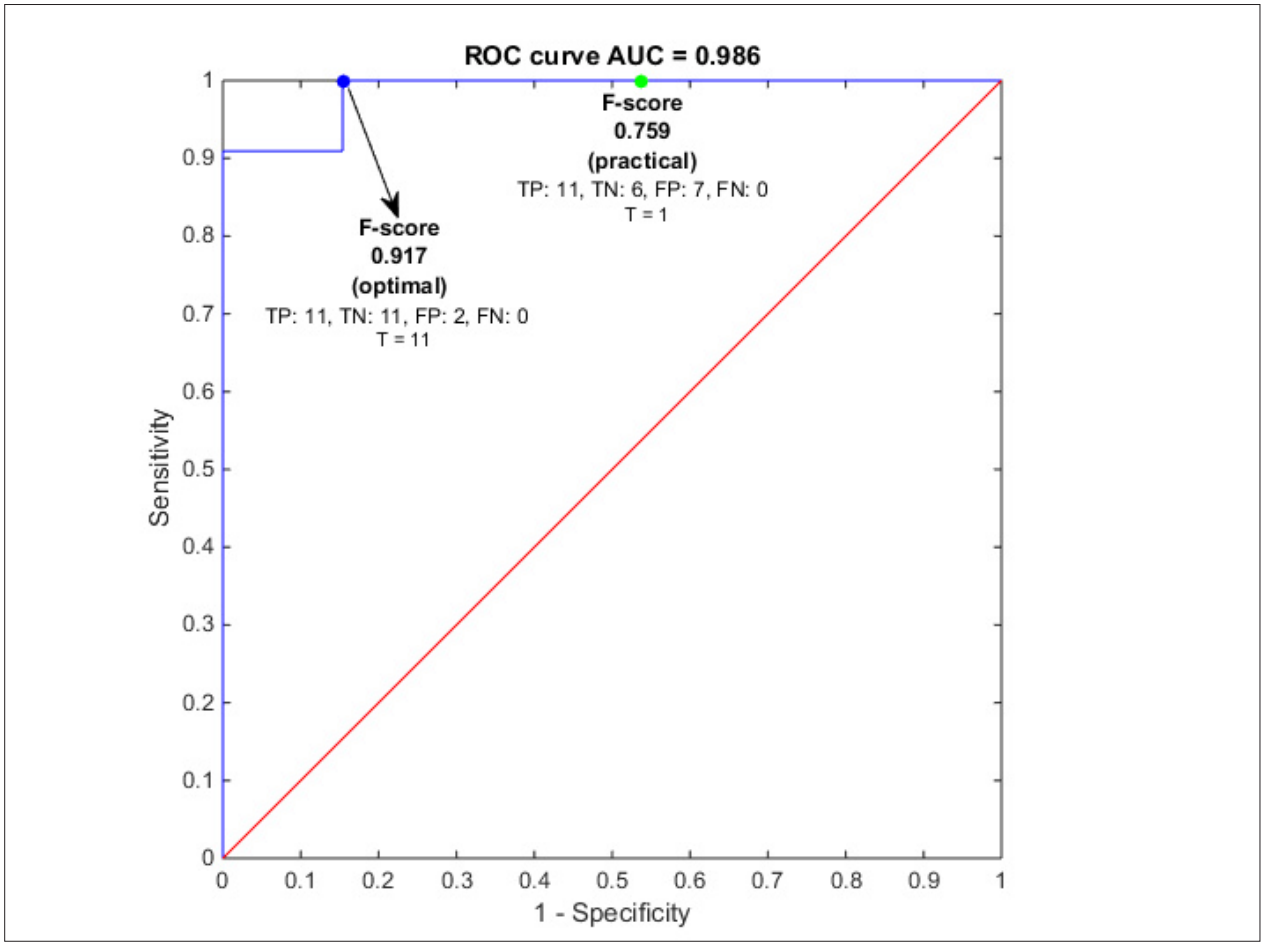


"Derived images obtained from the source. Reference image is used in color normalization methods. a) Intensity; b-d) RGB Channels; e-g) Color normalization methods (RGBHist, Reinhard and Macenko); h-i) Color Deconvolution Stains (Hematoxylin & Eosin)"

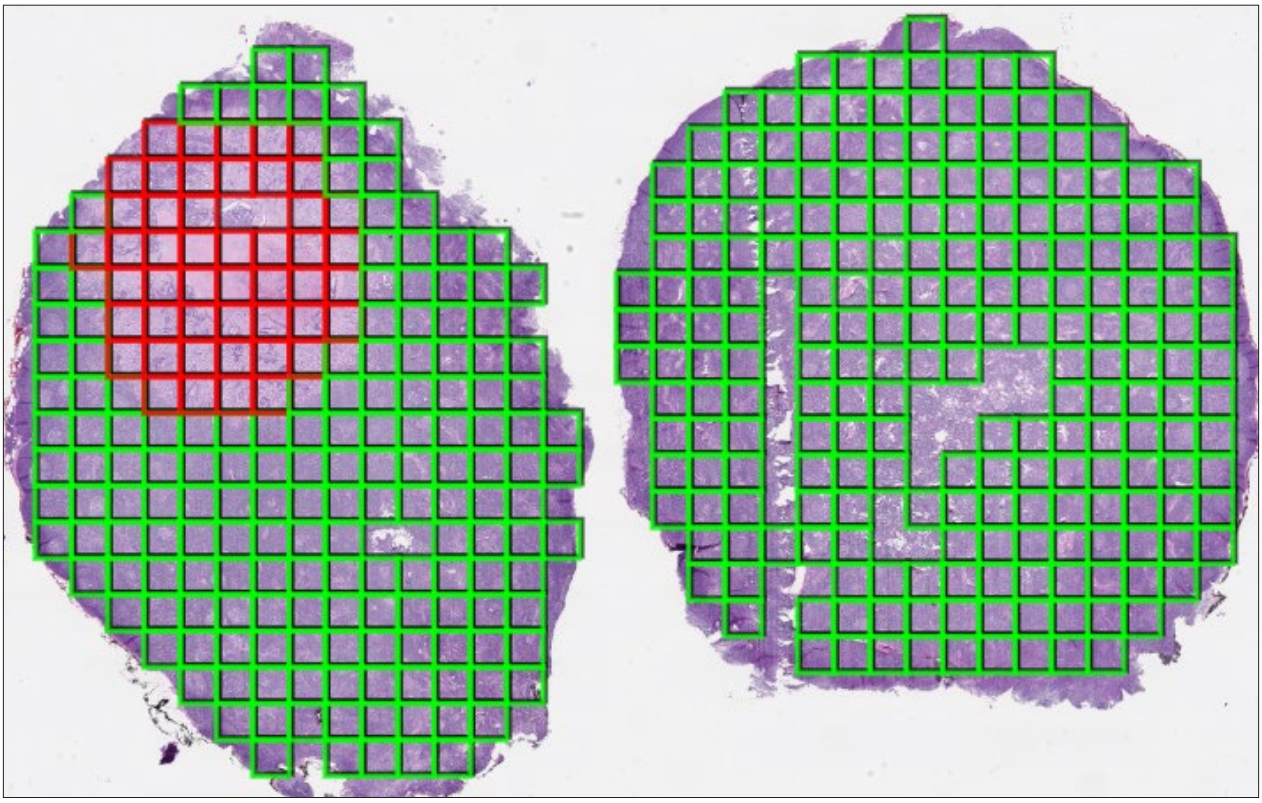
20x (0.45µm resolution). Twenty-eight of the LNs were identified by an expert pathologist as positive for cancer (P), and the remaining sixteen were negative (N). This database was divided into two groups. Group 1 (15P and 5N) was used for training and Group 2 (13P and 11N) was used for testing the classification technique. For all analysis each WSI was divided into non-overlapping 1000 x 1000 pixel sub-images that will be referred to as high-power fields (HPFs). For each LN in Group 1, at least one WSI was annotated by a pathologist to identify rectangular, HPF-scale regions as locally cancerous or locally non-cancerous. From these annotated slides, 924 HPFs (462 P and 462 N) were obtained. For each of these HPFs, statistical features based on gray-level co-occurrence matrices [1] and Law's texture energy measures [2][3] were extracted from 9 derived images [4]. The extracted features were submitted to a sequential forward selection (SFS) method [5] to select few non-redundant features providing best class separation (cancerous vs. non-cancerous region). Combinations of the selected features were tested on the 924 HPFs using k-fold cross-validation to find those that produced the best results and consequently to train our SVM-based classifier. In Group 2, WSI were not annotated for cancerous and non-cancerous zones on a HPF scale. Each LN, however, had been labeled by a pathologist as positive or negative for cancer. For each WSI, each section was divided into contiguous HPFs, and those which mainly contain fatty tissue, background, and tears were automatically excluded. Each selected HPFs was classified as cancerous or non-cancerous using the previously trained classifier to obtain the total number of cancer-classified per LN. A receiver operating characteristics (ROC) curve was traced by changing the discriminant threshold (T) used to label the LN as P for cancer as a function of the total number of cancer-classified HPFs

Results

During training, 5 Laws features were selected by SFS. Highly satisfactory k-fold cross-validation with a F-score of 0.996 ± 0.005 was obtained using only 2 statistical features computed at different scales. The ROC curve



"ROC curve and its Area Under the Curve (AUC). The blue and green points are respectively the optimal operating point (0.917 F-Score, 2 False Positive) and a more conservative choice (0.759 F-Score, 7 False-Positive). T is the discriminant threshold."



"Example of a test positive LN which was classified as positive and the classification at the HPF level."

obtained by applying the SVM-classifier to the test set is shown in the next figure.

Two valuable operating points can be identified which both guaranteed no false-negative. At T=11 we got 2 false-positives and an optimal F-score of 0.917, and with a more conservative approach, T=1, we got 7 false-positives and a F-score of 0.759. The top-left part of the slide displayed in next figure would have been proposed to the pathologist as the most suspicious region of the cancerous LN.

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SY09.01 | Quality Assessment and Quality Management

IMAGE QUALITY – REQUIREMENTS FOR CLINICAL AND RESEARCH APPLICATIONS

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Introduction/ Background

The digitalization of slides and subsequent utilization of whole slide images has a highly visible penetration within digital pathology workflows. Not only research applications moving towards daily use, but also clinical pathology applies digital images increasingly. The use of virtual microscopy, with all of their advantages and benefits, requires additional efforts to assure sufficient image quality [1]. This consist of multiple aspects to be fulfilled, such as sharpness, tissue completeness, color fastness primary and additional secondary properties like existence of artifacts, compression, availability of digitalization metadata and others.

Aims

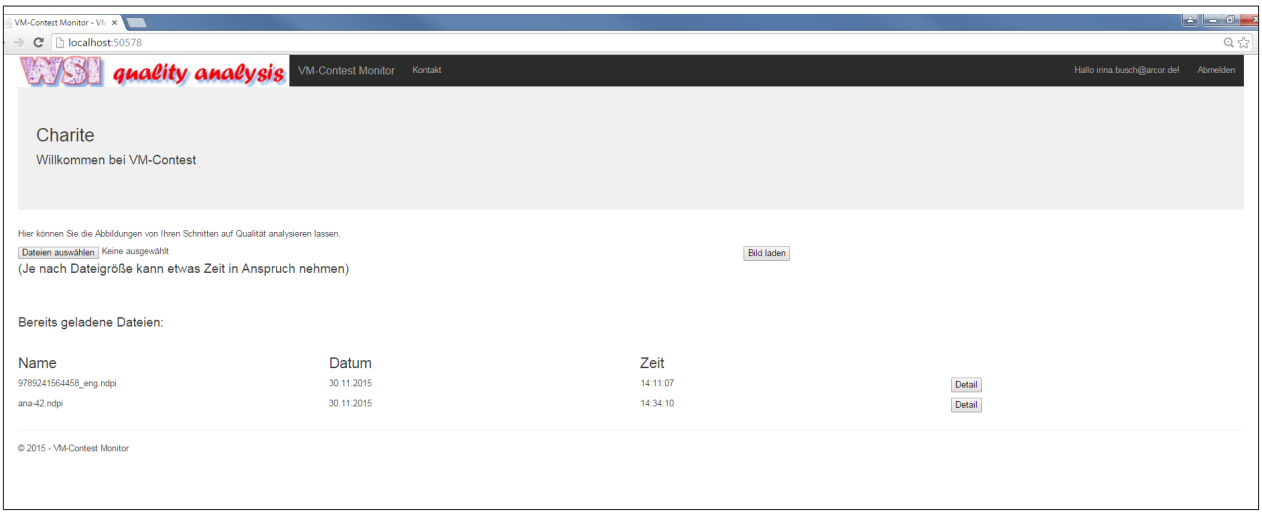
Scanning devices use multiple different technologies such as brightfield, laser or confocal. Moreover, they partly divide into patch and line scanning concepts. But not only hardware is constantly changing due to upgrades or further developments but also software algorithms, e.g. focus and stitching, are modified. This requires a standardized measurement algorithms and procedures to assure an appropriate quality for relevant aspects, depending on dedicated usecases.

Methods

Each dedicated requirement towards image quality has to be investigated separately, due to the fact that they differ in their origin. Distortions may be introduced through slide preparation, calibration of scanning device or even scanner software parameter. Therefore, we developed multiple algorithms and software tools to calculate a quality measure for each aspect automatically. Recently we are focusing on image sharpness [2], color fastness [3], [4] and completeness of tissue. Sharpness is measured by a no-reference focus algorithm per slide. Color fastness is calculated based on CIEDE2000 [5] for a reference IT8.7/1 color target mounted onto a glass slide per scanner. Completeness is automatically analyzed based on registration and comparison of whole slide overview image and preview camera data. Secondary quality parameters were not part of this investigation.

Results

We established a standard operation procedure to automatically apply slide based tests directly after digitalization. This enables scanning personal to execute quality inspection at a glance and schedule insufficient whole slide images for a rescan. Moreover, we made



"Prototype website to inspect quality aspects for uploaded slides. Each user can upload up to 10 slides."

some of these tools available as a web-based service including a based web-frontend with user management

This enables everyone within the digital pathology community to validate their slides, scanning devices and scanning parameter.

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BLUR QUANTIFICATION OF MEDICAL IMAGES: DICOM MEDIA, WHOLE SLIDE IMAGES, GENERIC IMAGES AND VIDEOS

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Introduction/ Background

We have designed a quality assurance tool to quantify blur by quantifying the sharpness of custom-sized tiles composing an image, generating global results, detailed exchangeable logs and sharpness-maps regardless of their dimensions, quantity or acquisition rate. [Ref1]

We have now integrated the programming libraries and the standalone program we built to existing workflows and software, in order to improve quality assurance procedures.

Aims

When integrated in an acquisition workflow, the ability to map the quality of local areas inside an image allows to re-acquire parts of the image that were not scanned properly, or discard and re-acquire images when the global results fall under the thresholds set by the users.

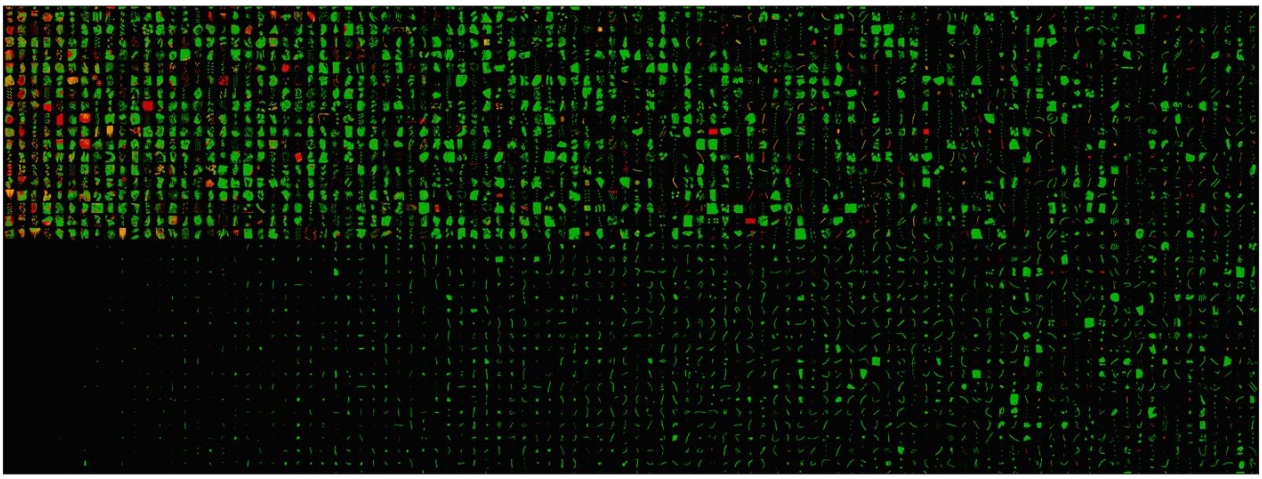
When integrated in an image analysis software, the regions of interest can be automatically chosen or suggested to the user according to the quality-map of the image to analyze, reducing the amount of incoherent analysis results.

When integrated as a library in an image management platform, or as a standalone program in a storage server, a systematic quality check can detect de-calibration of the acquisition software, failed acquisitions that may be automatically deleted and users can be notified to re-acquire the images when below their quality-thresholds profiles. A quantified quality score can be inserted in each image's metadata for traceability purposes.

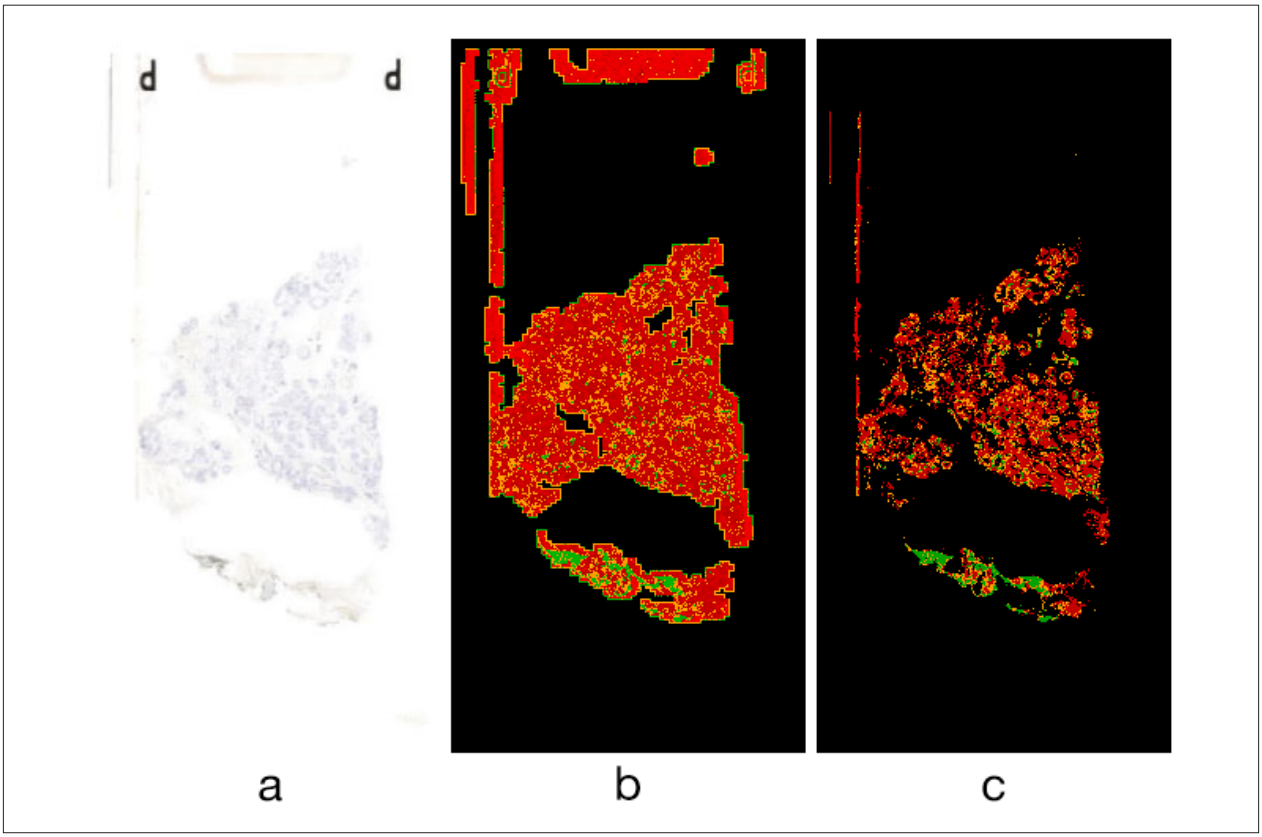
When integrated as a library in a local or remote image viewer, the best quality tiles can be sent first to the viewer, and magnification levels can be dynamically resampled for a better render.

Methods

One notable implementation, using our Java library, is inside the FlexMIm project [Ref2], which includes 27 pathology laboratories in the Paris area (coordinated by APHP), research laboratories from University Paris 6 Paris 7, as well as 3 companies: TRIBVN, PERTIMM and Orange. All Whole Slide Images are systematically analyzed and mapped as they enter the platform. The focus map may be displayed on the web interface next to the thumbnail link to the WSI, or in the viewer as a semi-transparent layer over the WSI, or over the WSI map.



"Automated tile-by-tile blur detection of 3954 WSI. Each pixel represents a 512x512 tile. Blurry tiles are represented as red pixels, partially blurry tiles as orange pixels, sharp tiles as green pixels."



""non-calibrated versus calibrated blur detection": the WSI (a) is analyzed with default calibration settings (b) or with dynamic calibration (c)."

During the test phase and first integrations in laboratories and hospitals as well as in the FlexMIm project, more than 5000 whole slide images of multiple formats (Hamamatsu NDPI, Aperio SVS, Mirax MRXS, JPEG2000 …) as well as hundreds of thousands of images of various formats (DICOM, TIFF, PNG, JPEG ...) and videos (H264) have been analyzed using our standalone software or our C, C++, Java and Python libraries.

Using default or customizable thresholds' profiles, WSI are sorted as "accepted", "to review", "to rescan". In order to target the samples contained inside each WSI, special attention was paid to detecting blank tiles. Dynamic blank tile detection based on statistical analysis of each WSI was built and successfully validated for all our samples.

Results

More than 20 trillion pixels have been analyzed at a 3.5 billion pixels per quad-core processor per minute speed rate. Quantified results can be stored in JSON formatted logs or inside a MySQL or MongoDB database) or converted to any chosen data structure to be interoperable with existing software, each tile's result being accessible in addition to the quality map and the global quality results. This solution is easily scalable as images can be stored at different locations, analysis can be distributed amongst local or remote servers, and quantified results can be stored in remote databases.

References:

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COMPARISON DISPLAY RESOLUTION ON USER IMPACT FOR DIGITAL PATHOLOGY

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Introduction/ Background

Digital pathology images are very large, up to 100000x100000 pixels which are 30 to 50 times larger than a radiological image for which 12 Mega Pixels (MP) medical displays can be used. Higher resolution displays may have an important influence on digital pathology ergonomics. Three displays with varying resolutions were studied to determine their impact on user interaction.

Aims

Our hypothesis was that "with higher resolution displays, pathologists need less interaction such as panning and zooming actions and can focus more on image content". A psycho-physical study has been carried out for validating this hypothesis at the University of Pittsburgh Medical Center.

Methods

Three experienced pathologists were selected. Seventy pathology including a wide variety of histological and cytological diagnoses were digitized (Aperio Scanscope XT scanner) and used in a previous study [Ava15]. Customized and optimized viewing software was used to display images and record pathologist's interactions such mouse clicks, zooming and panning. Three medical displays with different different resolutions were used: 2MP (BARCO MDSC-2124), 4MP (BARCO MDPC-4130) and 12MP (BARCO MDCC-12133), all with the same maximum luminance. Scripts were used for statistical analyse and 1D, 2D, 3D plotting results. User interactions with each image were used to recreate videos documenting of their exact navigation with each digital slide.

Results

The results of number of zooming and panning interactions are given in the Table 1, as well as averages.

When display resolution was increased, the number of panning and zooming interactions significantly de-

	number of panning interactions				number of zooming interactions			
display	obs1	obs2	obs3	average	obs1	obs2	obs3	average
2MP	1346	1589	581	1172	8421	18526	9999	12315
4MP	940	2139	819	1299	7090	23879	8235	13068
12MP	1015	1331	506	951	2409	3726	2405	2847

"Number of panning and zooming actions for all images, per observer and per display"

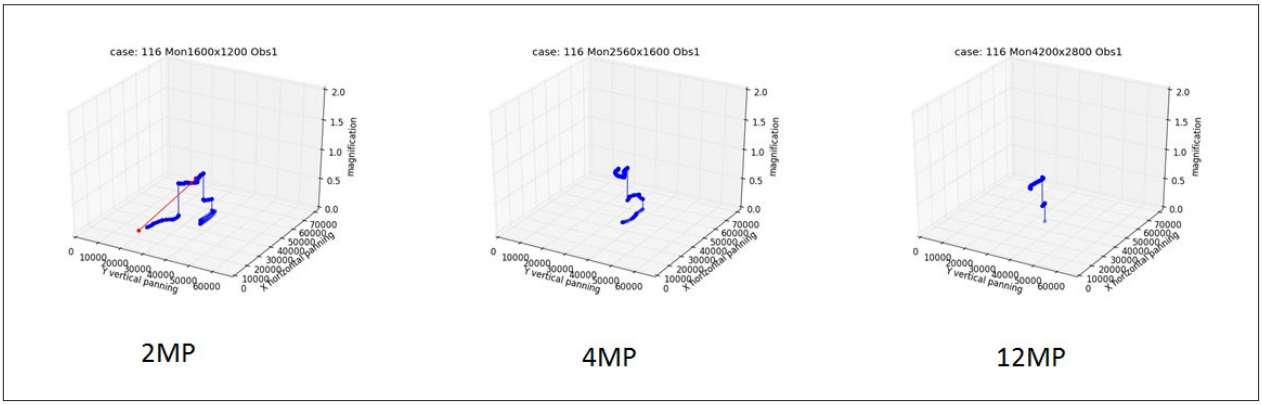

Figure 1: "3d navigation through the case labeled 116, for the observer 1 and the three monitors"

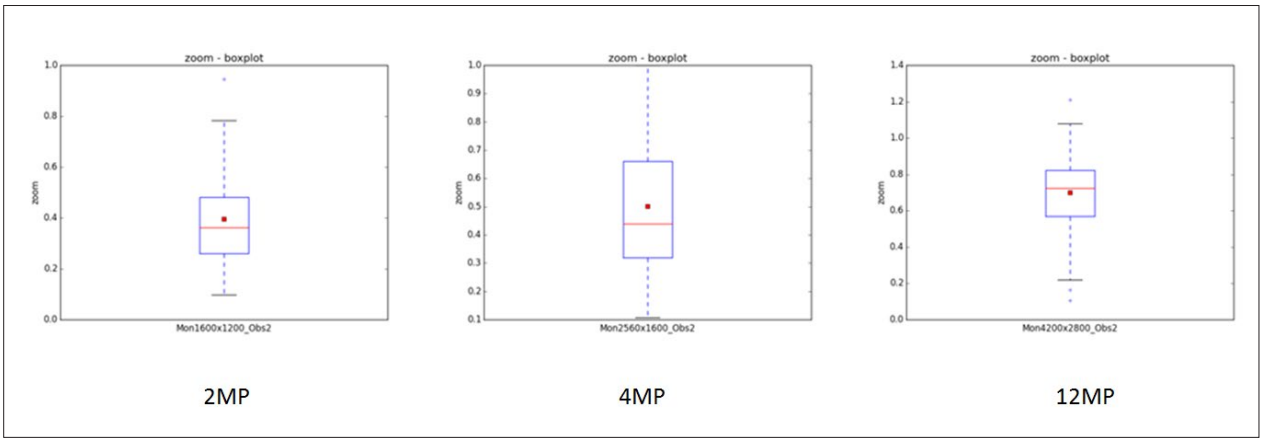

Figure 2 :"Boxplots of the zoom values for all cases on the three monitors for observer 2"

creases for all three observers. For panning, there was on average 1172 panning actions for the 2MP and 951 actions for the 12MP display. For zooming actions, there was on average 12315 zoom actions for the 2MP and 2847 actions for the 12MP display. Between the 2MP and 12MP displays, the ratio of the number of zooms was 4:1 in favor of the 12MP monitor. On figure 1, the 3D plots of one case for the three monitors show the navigation through the slide and show lesser points for higher resolution display. With higher resolution the pathologist goes more directly to the Region Of Interest (ROI) for making the decision. Figure 2 shows more analysis of the zoom values across the cases for the three monitors. The pathologists have the tendency to remain close to a value of 1 with the 12 MP display, where a value of 1 means that no zoom is applied. This is illustrated by the Figure 2 showing for observer 2 the boxplots of the zoom values for the three displays. It clearly shows that with higher resolution display the trend goes to get closer to one for the zoom value meaning no need to zoom in in the image.

We used three different displays instead of one unique display with three different resolutions. Though using just one display would have reduced variability of differing LCD panels, pixel size and structure, it would not have been commercially or clinically realistic.

Despite the limited number of pathologists, this study shows that display resolution used for digital pathology is important. Higher resolution monitors significantly help reducing the number of user interactions and thereby can minimize pathologist fatigue when reading digital slides.

References:

[Ava15] Ali R. N. Avanaki; Kathryn S. Espig; Sameer Sawhney; Liron Pantanowitzc; Anil V. Parwani; Albert Xthona; Tom R. L. Kimpe, (2015), Aging display's effect on interpretation of digital pathology slide, SPIE Medical Imaging

EFFICIENT, UNBIASED QUALITY ASSURANCE OF AUTOMATED TISSUE ANALYSIS APPLICABLE TO DAILY PATHOLOGY PRACTICE

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Introduction/ Background

Quantification of tissue biomarkers is increasingly demanded for diagnosis and is commonly performed by expert pathologists using microscopy of stained tissue at high magnification. This manual scoring is a reasonably fast, supervised procedure, but it suffers from inter- and intra-observer differences due to a) differences in selection of regions of interest, b) differences in quantity estimation, c) intra-tissue variability of biomarker expression.

Computers and whole slide microscopy scanners have made it feasible to perform high-capacity analysis of high resolution images of tissue. Image analysis (IA) enables better reproducibility, but conversely, the unsupervised analysis introduces challenges regarding accuracy. Furthermore, borderline cases will always have to be rigorously inspected by pathologists.

Many IA evaluation methods exist, but for pathology, a supervised comparison of experimental segmentation to an appropriately obtained standard criterion is the optimal strategy. The production of standard criterion necessitates evaluation of whole slide images to eliminate any possible region sampling bias while inter- and intra- observer bias can only be minimized by replacing any manual estimates by objective measurements.

A logical step is thus to change the task of the pathologist from quantity estimation to verifying the output an automated procedure reports. Still, verification of entire tissue slides is in daily pathology practice too time-consuming. To minimize the workload pathology is turning to stereological methods which aim to efficiently quantify matter unbiasedly and have been proved useful for supervised validation of automated analysis for Ki67 scoring of breast cancer. However, the workload still needs to be reduced to a level comparable to the manual scoring procedure.

Aims

We aim to enable high accuracy, objective evaluation of automated image analysis with a workload and workflow feasible for daily pathology practice. This regards both production of reference data for image analysis tool calibration and continuous quality control inspection of borderline cases.

Methods

This study investigates proportionator sampling, a very efficient stereological sampling scheme utilizing weighted sampling of regions of automated image analysis for manual evaluation of automated IA. The sampling of regions to be inspected by a pathologist draws upon the IA to assign probability weights to all regions. This results in a highly efficient, unbiased sampling and quality assurance estimate for the automated image analysis.

Results

Presented here is proof-of-concept of an efficient, unbiased image analysis evaluation methodology. The task of the pathologist is changed from quantity estimation to instead annotate discrepancies between the output from the IA and the tissue in a few sampled regions. From the annotations an unbiased quality assurance estimate of the IA can be estimated including levels of accuracy obtainable and expected workloads.

This confirms that the stereological proportionator sampling enables manual verification of automated whole slide image analysis for unbiased reference dataset creation and quality control inspection in borderline cases. Furthermore, the methodology is easily integrated into both image analysis platforms for production of reference data sets and laboratory information systems for daily pathology practices.

THE BENEFITS OF DIGITAL PATHOLOGY IN THE ASSESSMENT OF HER2 ISH IN A NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME.

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Introduction/ Background

In situ hybridisation (ISH) techniques, colorimetric (CISH) and fluorescence (FISH) have been widely applied in breast cancer and are valuable in confirming HER2 gene amplification status as a valid predictor of response to anti-HER2 therapies. UK NEQAS is a well-established multinational scheme for assessing the quality of laboratories performing HER2 ISH testing. Three years ago the UK NEQAS module for HER2 ISH testing moved from using cell lines to human breast cancer tissue samples to assess the interpretive accuracy and technical quality of laboratories performing HER2 testing by ISH. At this time UK NEQAS also began to provide participants with access to digital scans of haematoxylin and eosin (H&E)-stained tissue samples to facilitate interpretation of the ISH-stained preparations.

Aims

The objective was to evaluate the use of digital and telepathology in a National External Quality Assessment Scheme.

Methods

Up to 450 pre-labelled glass slides are sent to the HER2 ISH module tissue provider by UK NEQAS, 4 times per annum. The tissue provider selects a range of previously assessed breast cancer cases from their archives and mini tissue microarrays (TMA) are constructed consisting of 4 cases of breast carcinoma representing a range of HER2 ISH scores. During TMA block sectioning, every 25th slide is set aside for H&E staining and digital scanning. All unstained slides are returned to UK NEQAS by the tissue provider for distribution to the more than 200 international participants enrolled in the HER2 ISH module. To assist participants in the assessment of the HER2-ISH stained tissue sample they are provided with a web link to a digital pathology database (Philips Digital Solutions) where they can view the H&E-stained slide corresponding to their ISH-stained slide. The participants return the HER2-ISH stained slide to UK NEQAS for assessment and input the scores obtained for each of the four samples on an online database. The objectives of the HER2 ISH assessment are (1) to evaluate the accuracy of HER2 ISH interpretation by analysing the inter-observer variability in enumerating HER2, Cep17 counts and overall ratio scores and (2) to assess the quality of the ISH-stained section.

Results

Due to the large number of participants enrolled in the UK NEQAS HER2 ISH scheme, it is not possible to provide each laboratory with a H&E-stained slide. Without a reference H&E-stained slide it can be difficult to clearly identify invasive tumour cells on an ISH-stained preparation increasing the chance of error in the assessment of HER2 gene status in tissue samples. However, with the technological advancement of digital pathology, it has been possible to provide UK NEQAS participants with a relevant H&E-stained slide of the tissue samples. In ***Conclusion,*** digital pathology has greatly facilitated the move from cell lines to breast cancer tissue samples for the UK NEQAS HER2 ISH module. Access to the digitised H&E-stained slides has enabled participants to quickly and more accurately visualise and pinpoint areas of invasive tumour. Our experience confirms the significant role for this electronic resource in EQA, education and continuing professional development.

APPLICATION OF MEDICAL INFORMATION SYSTEM FOR IMAGE-BASED SECOND OPINION CONSULTATIONS – GEORGIAN EXPERIENCE

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Introduction/ Background

Medical information system (MIS) is at the heart of information technology (IT) implementation policies in healthcare systems around the world. Different architecture and application models of MIS are developed. Despite of obvious advantages and benefits, application of MIS in everyday practice is slow.

Aims

On the background of analysis of the existing models of MIS in Georgia has been created a multi-user web-based approach. This presentation will present the architecture of the system and its application for image based second opinion consultations.

Methods

The MIS has been created with .Net technology and SQL database architecture. It realizes local (intranet) and remote (internet) access to the system and management of databases. The MIS is fully operational approach, which is successfully used for medical data registration and management as well as for creation, editing and maintenance of the electronic medical records (EMR). Five hundred Georgian language electronic medical records from the cervical screening activity illustrated by images were selected for second opinion consultations.

Results

The primary goal of the MIS is patient management. However, the system can be successfully applied for image based second opinion consultations. The ideal of healthcare in the information age must be to create a situation where healthcare professionals spend more time creating knowledge from medical information and less time managing medical information. The application of easily available and adaptable technology and improvement of the infrastructure conditions is the basis for eHealth applications. It can be concluded, that the MIS is perspective and actual technology solution. It can be successfully and effectively used for image based second opinion consultations.

SY10.01 | **Standardization**

A REVIEW ON INTERNATIONAL GUIDELINES FOR DIGITAL PATHOLOGY

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Introduction/ Background

Pathologists are looking at digital pathology not as much as an efficient telepathology solution but as an instrument to improve the quality and efficiency of their own daily clinical work beyond the use of the conventional microscope. The term digital pathology (DP) should also include interoperability and workflow considerations, and basic technical aspects of whole slide imaging (WSI), messaging, and standards should be also known by pathologists.

Aims

The aim of this study is reviewing the technical features of WSI systems in existing guidelines available internationally.

Methods

The following guidelines and technical documents have been evaluated:

- College of American Pathologists (CAP), 2013: Validation
- American Telemedicine Association (ATA), 1999: Telepathology
- Digital Pathology Association (DPA), 2011. First WSI guideline
- Food and Drug Administration (FDA).
- Canadian Association of Pathologists (CAP-ACP).
- Centers for Medicare & Medicaid Services (CMS/ CLIA).
- Centers for Disease Control and Prevention (CDC/ CLIAC).
- Society of Toxicologic Pathology (STPath).
- European Commission (EU-EC).
- Spanish Society of Anatomic Pathology (SEAP-IAP).
- The Royal College of Pathologists (RCP).
- The Royal College of Pathologists of Australasia (RCPA).

Results

Since February 2015, a draft of Technical Performance Assessment of Digital Pathology Whole Slide Imaging Devices from FDA is available. Regarding display (monitor), a complete description of the display, graphics cards, and control software items to be considered are included (display technology [LED, LCD], color calibration, ambient light sensors,..) although there is no mention to refresh rate). In the College of American Pathologists (CAP) Digital Pathology Resource Guide, references to guidelines or position papers other than CAP guidelines are dispersed in other sections of this document. Hanna et al (2015) reviewed existing guidelines and position statements for digital pathology, with special attention to validation and telepathology. Evans et al (2014) made a comparison between American Telemedicine Association (ATA), Royal College of Pathologists (RCP) and Canadian telepathology guidelines. In summary we could find the following topics in the corresponding guidelines: System architecture was only mentioned in SEAP document. Components of DP system: ATA, DPA, FDA, SEAP, STPath. Badwith/Network: ACP, DPA, SEAP. Interoperability: ATA, ACP, CLIA, DPA, SEAP, STPath. Scanning speed: ACP, DPA, SEAP; Storage: ATA, DPA, RCP, SEAP. Image quality in WSI: ACP, DPA, RCP, SEAP; Image compression and processing: ATA, EU-EC, DPA, SEAP.

Conclusions:

- Some basic technical features of a WSI systems that are of high interest to pathologists are scanning speed (e.g. 1-2 minutes 20x), autoloader capacity (100 slides), image quality (resolution 0.25-0.50 µ/ pixel) rescanning rate (<5%), image compression (<70%), monitor quality (30 inches 4-8 MPx), and viewing experience (refresh rate, network bandwith).
- In primary diagnosis, we need some new regulations also in European countries

Acknowledgment:

This work has been supported by the AIDPATH project, an EU 7FP IAPP Marie Curie action, contract number 612471

OPTIMAL IMAGE DATA COMPRESSION FOR WHOLE SLIDE IMAGES

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Introduction/ Background
Whole slide scanning is rapidly entering routine pathology laboratories. Modern scanners enable digitization of tens or even hundreds of thousands slides each year. If all WSI images are stored permanently, hundreds of terabytes image files need to be stored. It is essential to use image storage methods that preserve the scan image quality, but also keep storage costs in a reasonable level.

Aims
Today all WSIs are stored with lossy compression methods using a variety of different file formats. At the practical level it is important to find an image file format, which results in small-sized image files but retaining image quality as "visually lossless".

Methods
In this study we compared file formats of Hamamatsu, Aperio, and 3D-Histech scanners to standard JPEG2000 and to JPEG2000 specially optimized for brightfield histology WSIs. As for image quality readout we used standardized resolution charts, and evaluation by three pathologists who ranked the images by their visual quality, when displayed on a 4K computer monitor.

Results
Differences in WSI file sizes of scanned images deemed "visually lossless" were significant. If we set Hamamatsu Nanozoomer .NDPI file size (using its default "jpeg80 quality") as 100%, the size of a "visually lossless" JPEG2000 file was only 15-20% of that. Comparisons to Aperio and 3D-Histech files (.svs and .mrxs at their default settings) yielded similar results. A further optimization of JPEG2000 was done by treating empty slide area as uniform white-grey surface, which could be maximally compressed. Using this algorithm, JPEG2000 file sizes were only half, or even smaller, of original JPEG2000. Variation was due to the proportion of empty slide area on the scan. We anticipate that wavelet-based image compression methods, such as JPEG2000, have a significant advantage in saving storage costs of scanned whole slide image. In routine pathology laboratories applying WSI technology widely to their histology material, absolute cost savings can be substantial.

ANATOMIC PATHOLOGY STRUCTURED REPORT UNDER FHIR

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Introduction/ Background

The last version of profile for the structured report of IHE was created 2011(IHE). Many discussions took place to improve and adapt the model of described document models for clinical practice. The working group developed xxx templates to cover e.g. the main tumor locations and the requirements of various organization about tumor documentation (e.g. CAP, national societies of pathology). The complexity of a Structured Report Document based on IHE and HL7 CDA structured increased. Up to now no German software vendor supports these templates directly. Another strategy was suggested by the German Working Group: the IHE should provide generic models usable for the other domains in pathology. But the general problem of missing implementation was not solved. The development of Fast Health Interoperability Resource (FHIR) began in 2012 and started a fascinating movement to prioritize the implementation over the theoretical correctness. The resources, documentation and examples are completely open and available under http://wiki.hl7.org/?title=FHIR .

Aims

In a first approach we started to "translate" the structured pathology report based on the requirements of the technical framework. The aim was to analyze the usage of FHIR resources for the Anatomic Pathology Structured Report, simplify the document structure and increase the flexibility of document handling.

Methods

At first a complete report was created based on the CDA-XML-structure and model of the IHE Anatomic Pathology Working Group. The data based on a real, but anonymous case (many thanks to Prof. Haroske, Dresden, Germany). If possible each segment of CDA report was mapped to FHIR resources. The FHIR report consisting of different internal and external document fragments (resources) was evaluated by a public available FHIR communication server (http://spark.furore.com/fhir) to validate and verify this document.

Results

A first FHIR based structured report was created and validated against a public available FHIR server (http://spark.furore.com/fhir). FHIR allows to create different document structures for any type of document : a document only with inside resources or a document with inside and outside (linked) resources. Our example consists of resources embedded in the main document file and linked resources. The FHIR document allows a great flexibility related to the document resources as well as data files. It is possible FHIR documents as XML, JSON (JavaScript Object Notation) or RDF (Resource Description Framework). Due to these various possibilities FHIR documents can be used in a web based application context easily.

COLOR CALIBRATION IN DIGITAL PATHOLOGY: THE CLINICAL IMPACT OF A NOVEL TEST OBJECT

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Introduction/ Background
Guidance from the Food and Drug Administration has recommended color standardization for whole slide imaging, as with all other digital systems. However there is known, unresolved and substantial variation in color between digital slide scanners.

To address this issue, we created a novel color calibration test object which uniquely utilizes histochemical stains and a tissue-like substrate, affording accurate internal calibration of WSIs.

Aims
We aimed to evaluate the clinical application of the novel test object.

Objectives included: 1. Whether calibration made WSIs appear closer in color to the glass slide counterpart, 2. Whether pathologists prefer calibrated WSIs and, 3. Whether calibration affected diagnostic confidence.

Methods
Six pathology cases that present known difficulties when viewed using a digital microscope were selected and WSIs were generated. These WSIs were calibrated using a color ICC profile created using spectral measurements from the test object.

Twelve pathologists, blinded to intervention, compared calibrated and uncalibrated versions of each WSI on a medical-grade monitor. The display was color calibrated using a colorimeter which accounted for ambient lighting.

Three, subjective responses were recorded on 7-point Likert scales.

Results
Calibrated WSIs were closer in appearance to the microscope in 40 of 72 trials, (56%) and were preferred in 46 of 72 trials (64%).

Calibration improved diagnostic confidence (median 6.00 vs. 5.00, $p=0.001$).

Diagnostic confidence with the calibrated slides was correlated with preference for color calibration ($r=0.499$; $p<0.001$).

STANDARDIZATION OF PATHOLOGY WHOLE SLIDE IMAGES ACCORDING TO DICOM 145 SUPPLEMENT AND STORAGE IN PACS

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Introduction/ Background

In recent years different technological solutions have emerged for the scanning or digitization of histological and cytological slides in pathology, from several manufacturers. High resolution scanning is usually based in tile (small fragment) or stripes (longitudinal areas) that are combined or stitched together to create a high magnification (usually equivalent to 20x to 40x magnification) global image. Thus, a large digital slide can be displayed using specific viewers to simulate the functions of a conventional microscope. A pyramid of images is a common solution. But each scanner manufacturer optimizes the process of collecting, managing and storing images in its own format, making difficult the interconnection between systems and the ability to share images between different formats, and, generally a heavy process of image conversion and a loss of information is needed.

Aims

The objective is to present the process performed on proprietary image formats of histological and cytological slides in pathology to convert them the be compliant with the Digital Imaging and Communication in Medicine (DICOM) standard, according to 145 and 122 supplements, and their subsequent storage in a Picture Archiving and Communication System (PACS).

Methods

Python was chosen as programming platform due to its versatility and available tools. Furthermore, for future projects, Python can be used to apply signal analysis on digital images. A Pentium Core i3 4GB RAM, 1TB server with Ubuntu 14.04.3 LTS Server was used. In the server, a developer platform Eclipse Version 3.8.1 allowed the installation of PyDev for Eclipse 4.3.0 and Eclipse Jgit 1.3.0 pugins. It has been connected to a Github repository to manage developing versions. The solutions has been structured into a Python package to obtain images coming from proprietary images to be standardized to an image compliant with DICOM supplement 145, and sending them to the PACS.

Results

In order to test compatibility, David A. Clunie's dicom3tools was used. This allowed verification of all Tags of the generated files, indicating those who are required according to each image, and also it offers information of those tags where included data does not match the standard and those with values that do not correspond to that standard. This tool helped to finally obtain a result of 0% errors in all generated files. Regarding storage tests, two different PACS were used. First, in collaboration with T-systems company, an open source dcm4chee DICOM Archive 2 (dcm4che.org), and second, in collaboration with IRE Rayos X company, a commercial IRE Store Channel 4.3 was also used in several tests. After some tests with commercial and open source PACS, we could obtain the following ***conclusions:*** In the negotiation phase, the PACS did not recognize the predetermined configuration stated in supplement 145. The solution was changing SOP to "VL Microscopic Image Storage". Each level of the pyramid will be stored as an instance inside the same series, and each tile as a frame inside a multi-frame object. The dcm4che.org PACS offers a WADO service that allows accessing each frame separately, inside the same object, which can be useful for the implementation of slide viewers in pathology.

Acknowledgment:

This work has been supported by the AIDPATH project, an EU 7FP IAPP Marie Curie action, contract number 612471

TYPING, GRADING, STAGING - THE ULTIMATE GOALS OF PATHOLOGY REPORTS MODELLED IN HL7V3/CDA

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Introduction/ Background
Clinicians want pathologists to provide their reports fast, understandable, and useable for clinical decision making. Reports without a concise diagnostic conclusion in terms of typing, grading and staging of a disease are of low value. Therefore Anatomic Pathology Structured Reports (APSR) should have those keystones also reflected.

Aims
Instead to define very long lists with specific TNM-formulas, grading values, and tumor classifications information models have been developed which allow a flexible use in the diagnostic reporting approach and a compact programming.

Methods
Exemplarily for any diagnostic conclusion a model for TNM- as well as ICD-O-classification of malignant tumors for HL7 reporting to cancer registries in Germany was modified and completed with codes and value sets by means of Art-Decor. Additionally, an assessment score model was developed applicable for a wide variety of grading systems used in neoplastic as well as non-neoplastic diseases. ART-DECOR is a toolkit allowing for maintaining information models designated to data exchange facilitating communication standards.

Results
An information model for TNM- as well as ICD-O-classification of malignant tumors for reporting to cancer registries in Germany supporting the required data set was developed and filled with appropriate codes and value sets by means of ART-DECOR. The IHE APSR Trial implementation with its templates and value sets was mirrored in ART-DECOR and completed by templates for TNM-, ICD-O-3-, and Assessment-Scores, based on the German HL7 Oncology Reporting requirements.

A model based approach for structured reporting of complex problems is the preferred approach instead of exhaustive lists of permutated single values.

Conclusion:
The developed templates are to be used as entries in the Diagnostic Conclusion section of IHE APSR allowing for a structured data exchange so that automatic clinical decision support is maximized.

SY11.01 | Clinical Workflow Integration

CHOOSING AND IMPLEMENTING THE CORRECT WHOLE SLIDE IMAGING SYSTEM

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Introduction/ Background
The whole slide imaging (WSI) market has developed enormously in the recent years. This evolution has made the selection and the implementation of digital pathology more complicated. Based on a validation process, we developed recommendations that need to be taken into account when implementing whole slide imaging. In addition to selecting the right hardware, one should also focus on laboratory organization, integration, training and handling.

Aims
The aim is to integrate WSI as efficiently as possible in a standardized process. This should lead to a user friendly workflow for pathologists and must Ensure the quality of the diagnosis.

Methods
We organized on-site demonstrations to test several available WSI systems with a fixed set of histology slides. We evaluated scanning quality, measured the scanning time, the amount of data produced and made a simulation of a routine workflow. We developed a to-be workflow beginning with the scanning process until the final diagnosis of the pathologist. The workflow focused on the integration of the system into the laboratory information system. We standardized the technical laboratory processes to increase the quality of the slides and the efficiency of WSI. The standardization included good labeling, adequate quality, standardized location and correct number of sections on the slide. We introduced a continuous workflow to reduce batch size and to decrease the turn-around-time. The pathologists were only allowed to work with WSI after training in order to manipulate the images as efficiently as possible. Finally we validated the system according to CAP guidelines for WSI for diagnostic purpose in pathology.

Results
It is very important to determine what the purpose of WSI implementation in the lab is. The different systems show large variations between them and not every set up will fit in the specific workflow of each lab. The workflow simulation gave us an idea of the scanning turn-around-time and made it possible to estimate the amount of scanners that would be necessary in daily routine. Another focus point is the total integration of WSI to find a synergy between the hardware, workflow and the way the system can be integrated into the IT infrastructure of the lab. Beside the bidirectional communication with the laboratory information system, data storage organization and its influence on the lab's productivity is also very important. Organizing the laboratory with a standardized continuous workflow will reduce the amount of data, increase speed and lower the amount of rescans. To prevent that WSI is used inefficiently, it is crucial to train the pathologists before they start using it. A lack of training will lead to a dislike of WSI due to poor knowledge of the available applications. The pathologists are indicating that it's crucial to have the correct hardware to manipulate the image in order to ensure diagnostic speed and to lower the threshold towards WSI. We may conclude that choosing and implementing the correct WSI solution needs a systematic approach to succeed on the long term. Defining and optimizing the workflow before implementing whole slide imaging is crucial. Hardware, workflow and IT infrastructure should match to ensure productivity. Training of the pathologists is decisive in order to ensure efficient use.

THE EFFECTS OF DIGITAL WORKFLOW SUPPORT AND WORKFLOW CONTROL FOR THE PERFORMANCE OF ROUTINE PATHOLOGY

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Introduction/ Background

Although the scanning techology for microscopic slides has been known for more than 15 years, its practical use in daily routine is still on the very beginning. Fast and reliable scanners enabled their increasing use in teaching, but not yet in consultation and primary diagnostics. So far the scanning is not handled as a process in the pathology laboratory by most of the pathology systems,leading to an interrupted workflow with delays and additional expenses. The requirement profiles for slide scanners can only be formulated with respect to their workflow integration.

Aims

The effects of different degrees of workflow digitalization have been studied as to analyze the sources of possible benefits of digital pathology as well as to identify the bottlenecks and inconsistencies in the workflow control in a routine pathology laboratory. The adherence to existing IHE Technical Frameworks has been evaluated, too.

Methods

Performance statistics of routine pathology were evaluated in different phases of digital workflow control over more than 10 years in a medium-sized institute of pathology.

Three phases were defined:

1. Uncontrolled, but digitally supported workflow with digital dictation, digital macrophotography, digital microphotograpy at few pathology Workstations, and a “classic”pathology software system
2. Digital workflow control including digital dictation and digital photography.
3. In a pilot study at the end of the evaluation period the additional benefits of slide scanning were estimated.

Results

In the period between 2006 and 2015 a decrease of turnaround-time of roughly 40% was seen. Alone the effects of a (sub)total digital workflow control contributed about half of that effect. The implementation of slide-scanning did not add further acceleration so far, but enabled some additional functionality for improving quantitative reporting. This was achieved without an explicit committment of the pathology software to standards in workflow control and with still leaving a few laboratory processes out of the control. Milestones and key elements of workflow management are reported in detail.

Conclusion:

All processes both in the laboratory and in the diagnostics have to be checked (and changed, if necessary) for being fit in a streamlined pathology workflow. The implementation of scanners into the routine diagnostics will enforce those essential development leading to increased productivity and quality.

INTEGRATION OF EXTERNALIZED DECISION MODELS IN THE DEFINITION OF WORKFLOWS FOR DIGITAL PATHOLOGY

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Introduction/ Background

The availability of digital pathology creates opportunities for the adoption of advanced workflow solutions focused on facilitating and improving the current way of working in pathology labs. Workflow-driven applications can help achieve increased efficiency and quality, support collaboration, and provide detailed insights into the lab processes. The implementation of workflow solutions also create effective means to monitor and measure activities, and to detect and solve issues. Our solution helps improve processes in the pathology lab (both with respect to efficiency and quality) by modeling and optimizing the existing workflows and by incorporating decision models for automatic execution of relevant tasks and path selection in these workflows. Examples of decision models relate to the implementation and automatic execution of protocols, detection of quality issues, and automatic evaluation of tests with image analysis to evaluate the need for pre-ordering additional tests.

Aims

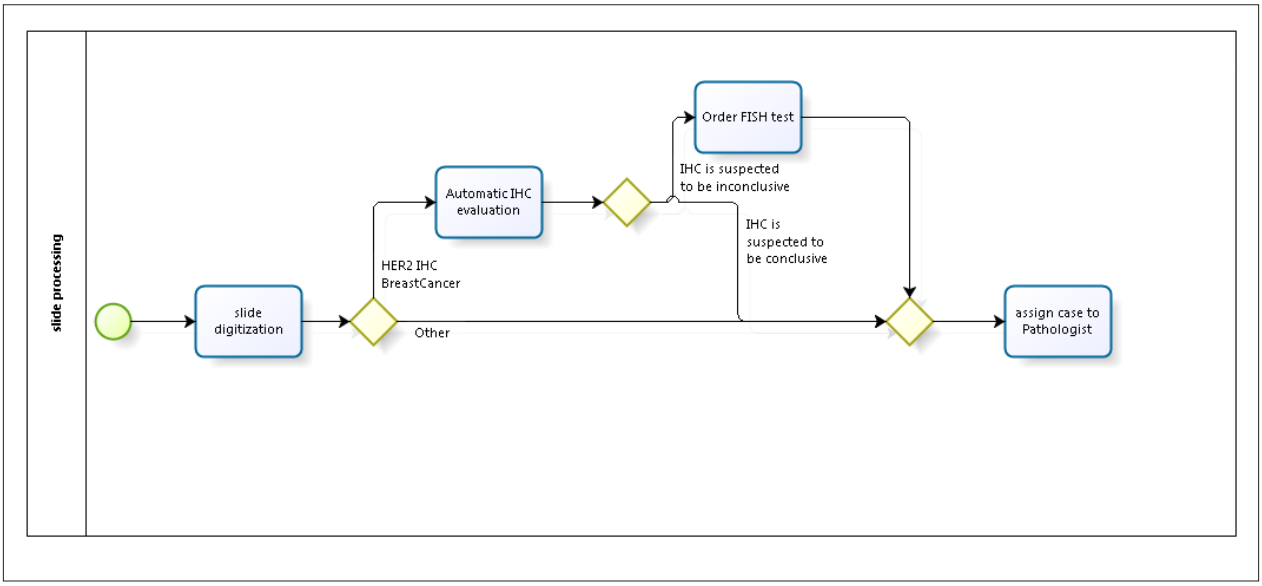
This work focuses on the modeling and optimization of relevant pathology workflows to enable clinical users to efficiently and effectively leverage the deployed digital pathology solutions for faster diagnosis and better patient outcomes. Next to identifying and addressing bottlenecks in the workflow, we aim to improve performance by enriching the workflows with clinical decision support.

Methods

We build a workflow-driven application enabling us to support and propose optimizations for pathology processes, while leveraging the availability of a digital pathology system. We select relevant workflows and identify opportunities for automating tasks and incorporating decision support. The selected pathology processes are represented according to the BPMN standard [BP] . We used the jBPM [jb] workflow suite (compliant with BPMN 2.0) for the modeling and execution of the processes. Programmatic tasks in the workflows are linked to external services executing the logic required by the tasks.

Results

We proposed a workflow solution enabling the representation of decision models as externalized executable



"Integration of decision models in workflow"

tasks in the process definition. Our approach separates the task implementations from the workflow model, ensuring scalability and allowing for the inclusion of complex decision logic in the workflow execution. In

we depict a simplified model of a pathology diagnosis workflow (starting with the digitization of the slides), represented according to the BPMN modeling conventions. The example shows a workflow sequence that automatically orders a HER2 FISH when IHC is borderline according to defined customizable thresholds. The process model integrates an image analysis algorithm that scores images. Based on the score and the thresholds the decision model evaluates the condition and recommends the pre-ordering of an additional test when the score falls between the two thresholds. This leads to faster diagnosis and allows to balance the costs of an additional test versus the overhead of the pathologist by choosing the values of the thresholds.

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THE ROLE OF THE TECHNICIANS IN THE DIGITAL PATHOLOGY IMPLEMENTATION. SEARCHING OPTIMIZATION.

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Introduction/ Background

Scanning histological or cytological preparations is a crucial element in the process of digitization of Pathology Departments, along with the traceability of tissue samples and the reports management. The scanning time and the high size of the files are still considered suboptimal for full implementation.

Aims

In order to optimize time and space a comparative study in our center has been carried out.

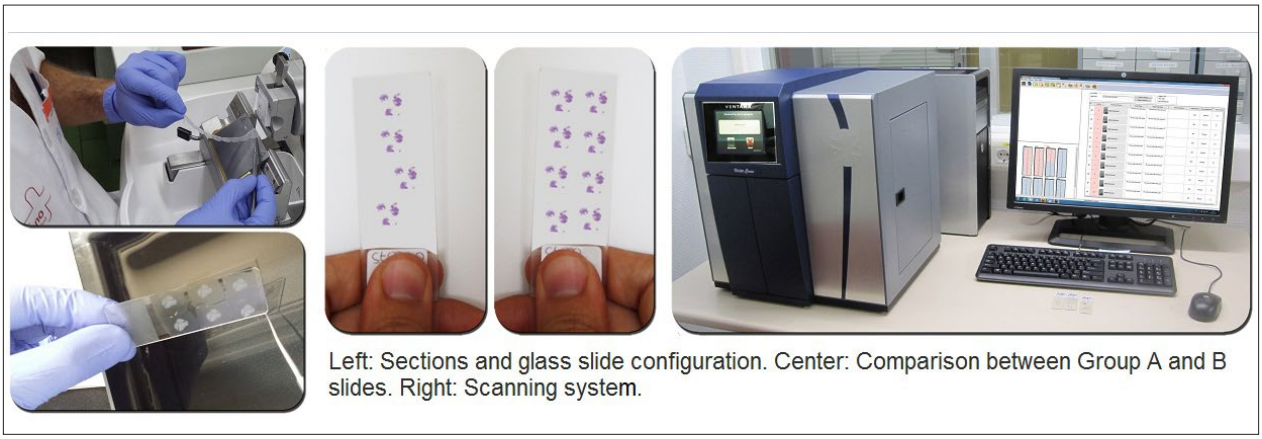
Methods

A total of 25 endoscopic samples (5 esophageal, 5 gastric, 5 duodenal, 5 colonic inflammatory, and 5 colonic neoplastic) were selected with the intention of comparing different parameters (scanning time, error rate during scanning and hard disk storage) between the original histological glass slides (group A: 2 slides per case, 50 preparations) and new sections, with levels grouped into a single slide (group B: 1 slide per case, 25 preparations). They were scanned at 20x magnification in routine way using the Ventana iScan scanner Coreo (Roche diagnostics). The process was repeated 4 times to calculate averages.

Results

The average scanning time was 5 hours 40 minutes (6m 48s / slide) in group A and 5 hours 10 minutes (12m 24s / slide) in group B. The error rate was higher than it had been found in previous studies (2-3%) with 6% errors in group A and 3,9% errors in group B. The space occupied on the hard disk was 11.87 GB in group A and 9.6 GB in group B (475 MB/case vs 385 MB/case, respectively). The average number of tissue sections per case was 7 in group A and 8 in group B.

Conclusions: There is a clear benefit of standardizing and optimizing the number of cuts per slide in terms of storage (saving 19%), biopsy sampling (14% more tissue) and error rate (35% less), including a not negligible decrease in the scanning time (9%) in the study conducted.



Left: Sections and glass slide configuration. Center: Comparison between Group A and B slides. Right: Scanning system.

"Comparative study: searching optimization"

SOLUTION FOR THE OPTIMIZATION OF PATHOLOGY CASE DISTRIBUTION LEVERAGING FLEXIBLE DEFINITION OF POLICIES

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Introduction/ Background

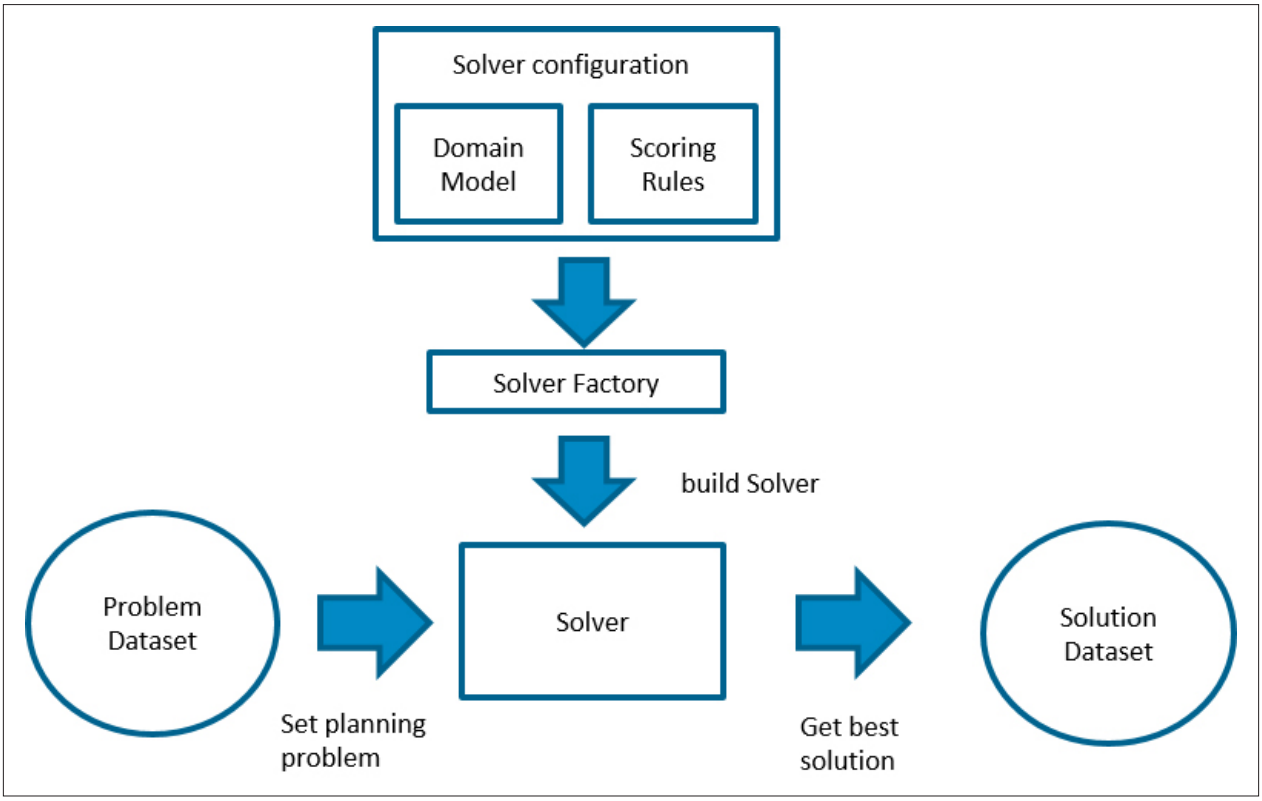
The adoption of digital pathology has the potential to enable significant workflow improvements leading to increased efficiency–in terms of better utilization of resources, higher throughput and lower turnaround time of cases–, and more effective collaboration. Streamlined workflow solutions make it easy to monitor both performance and quality, and help avoid errors. The policies driving the distribution of cases to pathologists (dispatching) have a large impact on the throughput and turnaround of cases in a pathology lab. Leveraging the availability of digital pathology we develop an application focused on the management of worklists of cases and their automatic dispatching for diagnosis. Our solution includes the modeling, simulation and optimization of the dispatching policies and their adaptation.

Aims

We develop applications to enable clinical users to leverage a digital pathology system for increased efficiency and better patient outcomes. The work addresses information integration requirements, and aims to identify and propose solutions for performance bottlenecks in existing processes. A process with potential for improvement is the case distribution to pathologists for diagnosis.

Methods

We implemented key components enabling to manage and retrieve case and pathologist information, to propose an optimized assignation of cases, and to visualize worklists and assign cases to pathologists. **Worklist visualization**: Provides an overview of(active and completed) cases with relevant information (e.g.



"Building the solver"

status, number of slides, organ, clinical question). The tool also shows for each pathologist the assigned and diagnosed cases, specialties, deadlines, etc. **Dispatching optimization module**: Proposes assignments based on case features (e.g. type, complexity, average diagnosis time) and on pathologist characteristics (e.g. specialty, available time) [j] . Aims at optimizing user-defined goals, such as the pathologist time and the turnover of cases. We use the OptaPlanner package [OP] of jBPM [jb] and define the domain model of the problem and the scoring rules according to policies based on the requirements of the clinical users.

depicts the process of building and executing the solver to generate a suitable dispatch solution. **Services for data management**: Allow to retrieve the relevant metadata of incoming cases and the agenda information of pathologists.

Results

Our case distribution application supports both the manual dispatching of cases to pathologists and the automatic assignation according to defined policies. The optimization component applies the policy models to send cases to pathologists for diagnosis. The schedules are generated according to the optimization goals, e.g. to improve throughput or turnaround. The configuration can be customized to apply dispatching rules and optimization goals specific to each deployment site. The visual application provides insight into the status of cases and allows users to change the assignation of cases when needed (e.g. when agenda changes occur and cases need to be reassigned).

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THE IMPACT OF INTRODUCING VIRTUAL SLIDES AS A REPLACEMENT FOR POWERPOINT PRESENTATIONS IN THE STUDENTS' MICROSCOPY LABS

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Introduction/ Background

The medical school students in Timisoara, Romania have been studying pathology slides in microscopy labs according to a protocol which uses classical PowerPoint presentations as guides for understanding the microscopic features of diseases, followed by individual examination of the glass slides under the microscope.

Aims

We aimed to assess the impact of replacing those presentations with virtual slides (VS).

Methods

In the middle of the semester, for the benign tumors microscopy lab, which is presented over the course of 2 weeks, we used 3 VS, while the other 3 slides were presented in the classical PowerPoint manner. All attending students from the 3rd year of the Medical School of the University of Medicine and Pharmacy "Victor Babes" Timisoara were asked to fill out an anonymous questionnaire at the end of the lab, in which they graded the difficulty in identifying lesions, chose the best/least understood lesion and pointed out the best manner of presentation.

Results

431 valid questionnaires were collected. 52.9% of the students indicated one of the 3 VS as the best understood lesion, while 59.62% chose a different VS as a least understood one. One VS was also the top best (113/332 votes) while another the least understood (34/126 votes) lesion. 74.01% students agreed that VS helped them understand the microscopic criteria better, while 74.71% would like VS to be used in the labs to come.

Conclusion: VS were appreciated by the students as a novelty and a more impressing way of studying pathology slides, but did not dramatically improve the easiness with which they identify and understand the lesions.

Work supported by the project „Studiu multicentric privind utilizarea abordului robotic în corelarea scăderii nivelului de adipokine circulante din obezitate cu riscul apariţiei cancerului pelvin < ROBOCAPE >" - ID 1846 / Cod SMIS 48478 - POSCCE

DEVELOPMENT OF AN ANDROID BASED INTERACTIVE GUIDE FOR THE BERLINER MEDIZINHISTORISCHES MUSEUM DER CHARITÉ

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Introduction/ Background

Pathology is the science of diseases that ranges from macroscopic to histologic, and of course molecular changes. To offer a holistic education we wanted to involve portable electronic devices to combine information on diseases with microscopic changes and formalin fixed organs (macroscopic preparation).

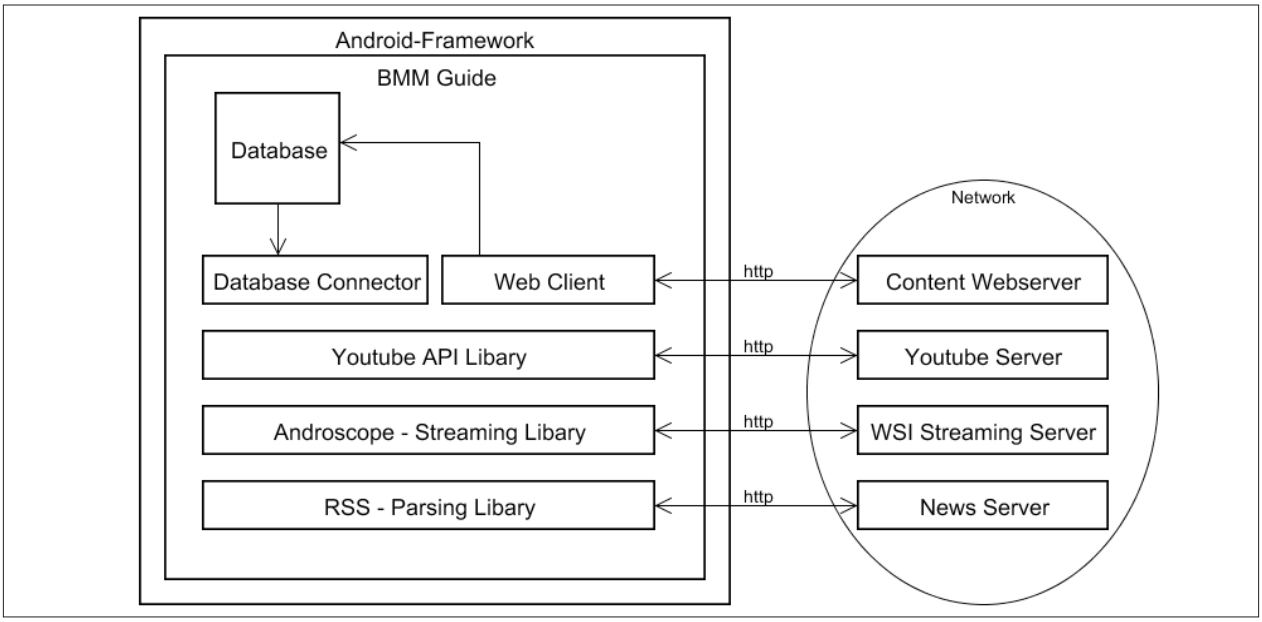
Aims

At the time of compilation of this application there was no alternative, useful solution that offers the possibility of extensions towards virtual microscopy. Moreover, other solutions always use fixed databases or do not provide tools for content updates. Hence, it was required to create an appropriate system. Additional aimed feature are high performance, data-caching and the opinion to use the app in offline mode without a network connection. By the reason of the large amount of smartphone and tablet computer that runs the Android operating system and cheaper devices this platform was used.

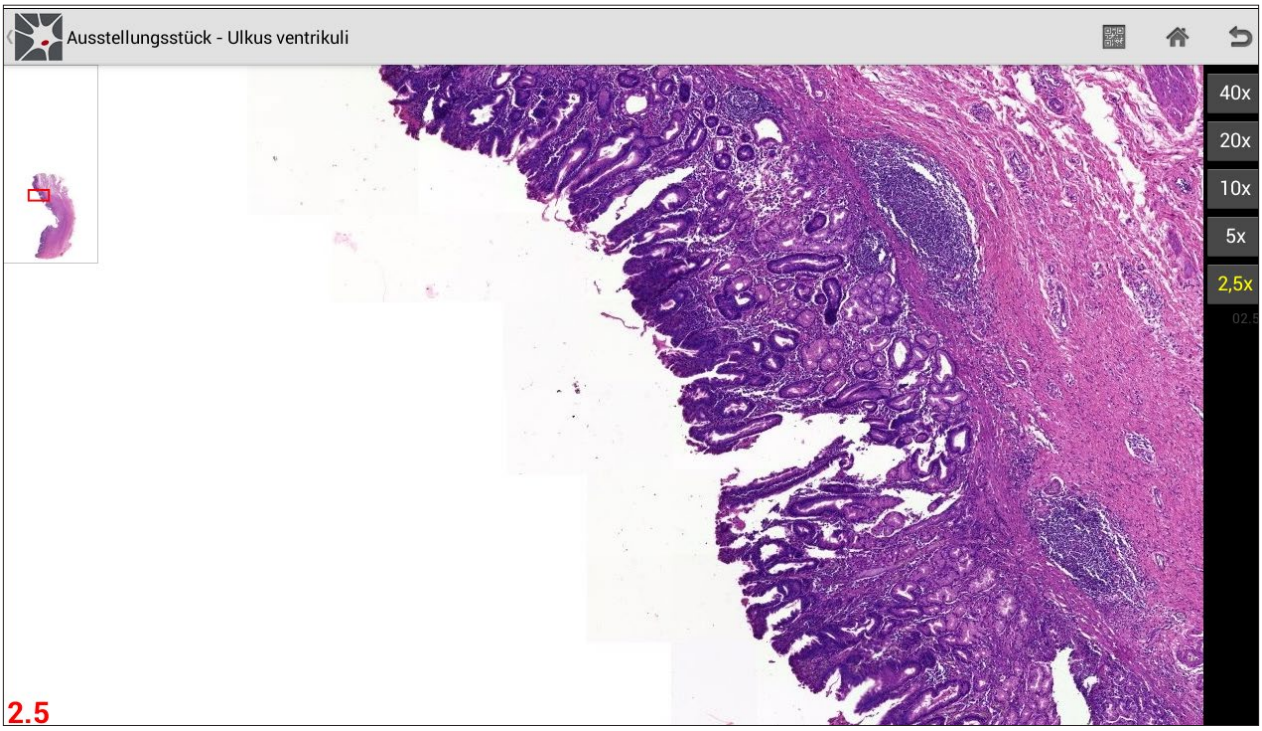
Methods

We combined our virtual microscope „AndroScope“ [1] with a new developed user-interface of the „Berliner Medizinhistorisches Museum“(BMM) for android based mobile devices such as smartphone and tablet computer. As a content we used images of the exhibition samples, informations on the corresponding organ and disease, as well as the epidemiology data and whole slide images for visualization of histological changes. Linkage of digital content and samples is realized using QR-codes to assure valid and user-friendly recognition. We have also evaluated other technologies such as NFC, Bluetooth, WiFi or GPS to ensure that the QR-Code solution is the best opinion [2]. The application offers an online mode with full functionality and an offline mode with limited access to images as well as to the virtual microscope

The application main database is stored local on the android device and online update capabilities were added.



"System architechture"



"Virtual microscope embedded into BMM guide displying slide for recognized exhibit."

Results

The "BMM Guide" is available for all visitors of the museum on lendable devices or for students (professional audience) using their personal devices and installing the application manually via the web-access eduroam. The guide is connected to the internet. It is designed to easily expand, update or transfer the content catalogue data. At the moment there are connection between the exhibit and text-, image-, video- or virtual microscope content via QR-Code. The offline mode is limited to the connection between text content and the exhibit. We also implemented a multi-language support for English and German. The application have information like room plans, opening times and latest news of the museum. The museum guide is an easy handable, selfexplaining blended learning tool, that can be embedded in the general education

.This guide for the exhibitions of the Berliner Medizinhistorisches Museum opens a new branch for self-study of students. Nevertheless he still has a potential to be integrated in curricular lectures in the future.

"Welcome screen of the BMM guide."

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OPEN ACCESS PUBLICATION IN PATHOLOGY – ADVANTAGES, CONSTRAINTS AND NEW TOOLS

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Introduction/ Background

Open access journals are financed by authors (i.e. research grants), rather than by readers or subscribers. This business model allows a world – wide uncontrolled distribution of medical information and scientific knowledge. The financial of shifting the finance and the opportunities for misuse raise significant concerns regarding non – scientific impact and article content integrity.

Aims

Herein we report and discuss ideas and experiences of open access publication focusing on diagnostic pathology.

Methods

Our experiences are drawn from the open access online journal diagnosticpathology.eu. The journal offers the opportunity to publish case reports "beside the microscope" and to submit data for "interactive publication". Both tools are unique, and cannot be found elsewhere. For publication of suitable articles, we demand the submission of glass slides, which will become completely digitized (virtual slides, VS).

Results

The journal is online since ten months, and the only completely independent open access journal in medicine. We have published several case reports under the headline "How do I diagnose…?" The presented form offers a guide through the article and permits a complete publication "besides the microscope", commonly in less than one hour. Automated links to reference search items are included as well as virtual slides. The strict publication format permits fast submission of unique or interesting cases, and, in addition, the implementation of the publication into a case – related open and flexible image date bank.

Conclusions: The mandatory inclusion of virtual slides is a unique quality control. The journal diagnosticpathology.eu is embedded in a cloud that will consist of an archive of published cases with virtual slides, an express review forum with a corresponding duty plan, an automated measurement system of histological slides, and open access atlases such as hazards of natural and artificial fibers (fine granulate) and a collection of all known pulmonary diseases.

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CYTEST - A NEW PLATFORM FOR TRAINING AND TESTING IN CYTOPATHOLOGY

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Introduction/ Background

Clinical training at the European level requires flexible ways to provide education across borders with the goal of a unified way to teach and assess quality. The CyTest project focuses on Cytological Training at European Standard through Telepathology. The project (2014-1-IT01-KA202-002607) has been approved and funded in 2014 by EU within the ERASMUS+ Program. The project consortium is composed of 4 leading university Institutions (COREP and Universitiy of Turin, University of Padua, Imperial College of London, IP-ATIMUP/University of Porto and University of Graz) with technological development and support provided by CRS4. In addition, it benefits from the collaboration of International Organizations (EFCS, Eurocytology, OME) and is open to contributions from additional groups and Societies.

Aims

Our aim was the establishment of a platform for the sharing of digital pathology images and of an auxiliary system that will use the latter platform for the distribution of cytologist training courses with an integrated virtual microscopy capability.

Methods

The CyTest platform is based on the integration between Moodle, an e-learning platform designed to create personalised learning environments, and OME OMERO, a well known open source software for visualization, management and analysis of biological microscope images. The former is used to provide access to a database of questions produced by specialized trainers and the latter provides access to digital pathology images and related metadata. We chose to base our infrastructure upon Moodle because it is one of the top leading platform for online education with a large community of users across both academic and enterprise level, highly customizable and modular. OMERO was chosen because of its compatibility with a large number of image formats for digital pathology images, its handling of image metadata (i.e., TAGs and Regions of Interest) and its easily extensible web platform.

Results

The web platform can be used with a wide range of devices, it is compatible with most of the image formats produced by digital slides scanners and it can scale to a wide student body. Teachers can create courses, populate the Question Bank and aggregate questions in quizzes, while students can take classes and tests. When creating questions, teachers can choose images previously loaded and annotated. We provide two new types of questions: multiple choice, focused on an image and its ROIs, and interactive, where students identify areas on the image by markers that will be automatically compared to instructor's specified ROIs. The currently deployed system holds already a set of several hundreds of images classified by categories (e.g., tissue type and diseases) with associated ROIs identified by pathologists. The CyTest platform provides a full technological solution for a more homogeneous training and testing of cytotechnicians and cytopathologists with uniform quality level assurance mechanism. The system could be easily extended to support the teaching of histopathological diagnosis. Moreover, the CyTest platform paves the way to an e-QUATE test, thus providing an efficient and economical way to replicate the test at European scale. The sustainability of the platform and the supported educational material (images, questions and evaluation algorithms) will be guaranteed by its integration in EFCS activities. We expect to distribute the CyTest System for validation by October 2016, for further information contact infocytest@corep.it.

COMPARATIVE ANALYSES BASED ON WSI VIEW PATHS RECORDED DURING MULTIPLE PRACTICAL EXAMS IN ORAL PATHOLOGY

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Introduction/ Background

Data collected about the ways students view Whole Slide Images (WSIs) during their practical exams can be used for many interesting analyses. Tracking such viewing behavior over years enables multiple comparisons and leads to drawing more general conclusions about observed viewing patterns.

Aims

First goal of this work was to collect data about how students view WSIs attached to questions during practical exams in oral pathology conducted over several years. What is important, we were collecting this data for all or for most students participating in the exams. Second objective was to analyze the data using specially prepared methods. This way we could gain interesting insights into students' viewing behavior. Finally, by analyzing data from a few exams we wanted to compare the observed viewing patterns across multiple years, and find general conclusions which hold true for multiple exams.

Methods

A scalable software-based view path tracking method with centralized database was used to collect data describing how students pan and zoom across WSIs attached to exam questions. The tracking software was active during multiple practical exams in oral pathology conducted in the recent years at Poznan University of Medical Sciences in Poznan, Poland. Data about over 100,000 view fields has been gathered, and we used it in various analyses, including visualizations and numerical calculations. The latter were based on computation of per-view-path metrics, like number of view fields, viewing time, average zoom level, focus on a region of interest, dispersion. Generated overview images and calculated numbers were compared and aggregated for different students, questions, student classes, and exam years. On each level, we split the data into groups of students who answered a question correctly, and those who gave an incorrect answer. We looked for correlations between the calculated metrics and answer correctness, and even attempted to predict students' answers based on the metrics, using machine learning approaches.

Results

The view path tracking implementation has successfully collected data about WSI areas viewed by students during multiple practical exams in oral pathology, and we were able to use this data for multiple analyses. Produced visualizations (static images and animations) provided clear overviews of how individual students viewed WSIs, and which areas of the slides were most often viewed when answering correctly or incorrectly. Calculated metrics enabled more objective comparisons, and aggregation of obtained numbers resulted in finding more general patterns. We found that students who gave incorrect answers tended to view the WSIs for longer time, go through more areas, often more dispersed across a slide, and focus less on the expected region of interest. Analysis of data split by student classes taught by different assistants helped in assessing personal impact of a teacher on his or her students' results. Finally, thanks to the view path tracking data collected over multiple years, we were able to compare results of the analyses from different exams, to see whether the observations hold true for multiple groups of students and for a longer period of time. This way we found certain consistencies and patterns reoccurring over years, which makes such findings particularly meaningful. Yearly analysis also helped in assessing didactic value of used slides and identifying slides which potentially require more attention during oral pathology classes.

VIRTUAL PATIENTS AND SERIOUS GAMES IN MEDICINE

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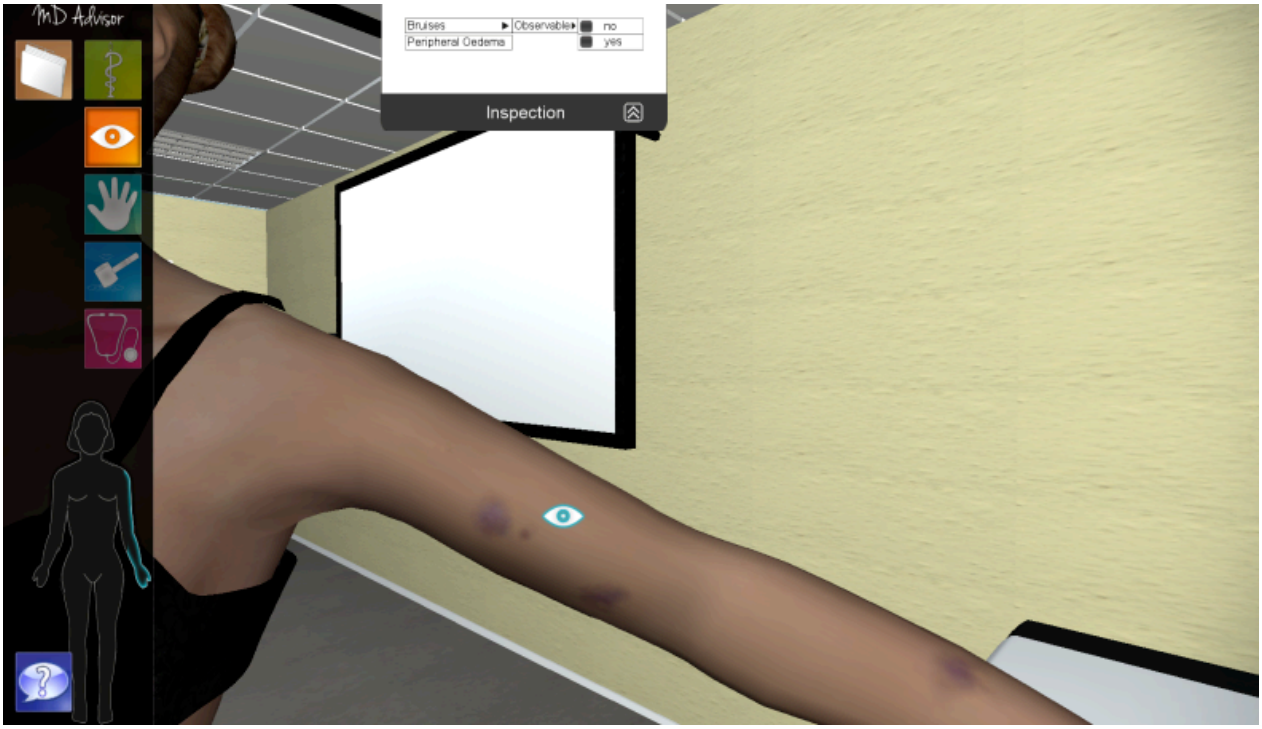
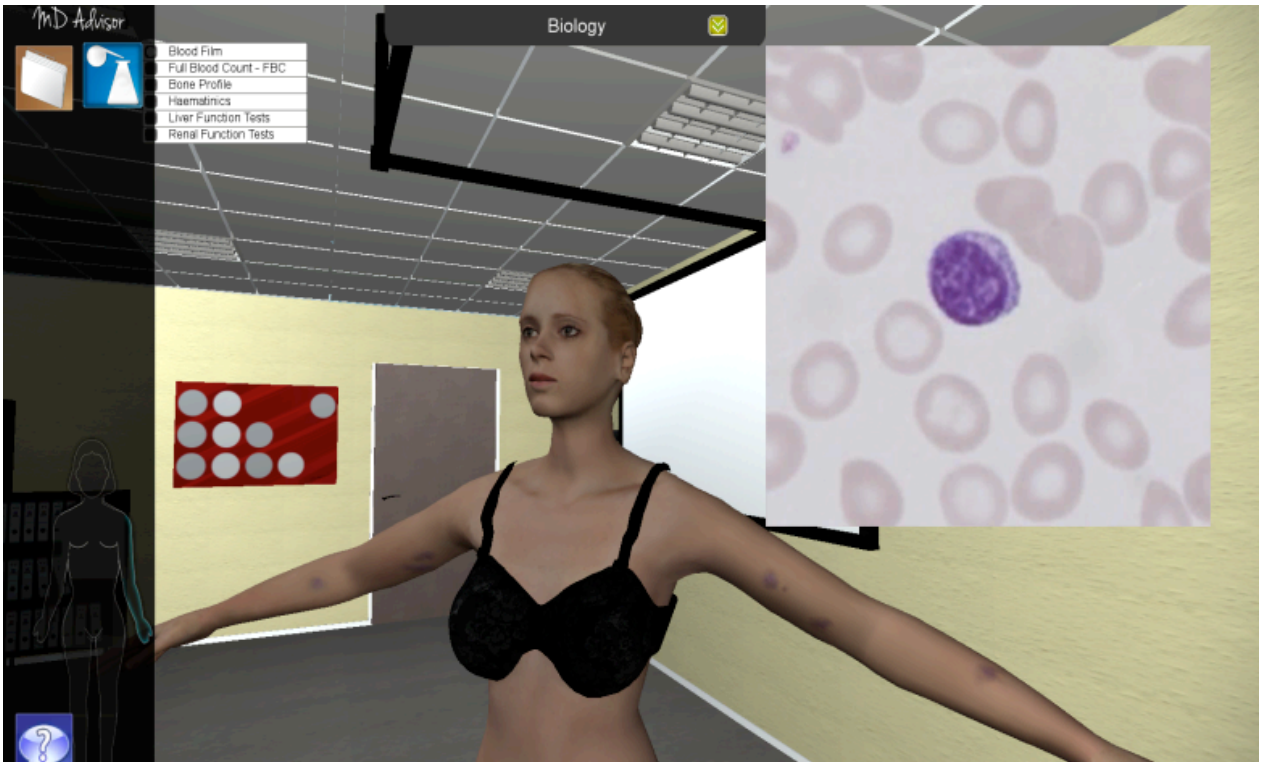
Serious games (SGs) are immersive learning and training tools. They have gained a widespread interest worldwide and are gaining ground with respect to formal education. Most serious games follow a learn-by-doing pedagogic principle. One of the main advantages of this approach resides in the fact that people retain 90% of what they do as opposed to 10% of what they read [1]. It is also a very efficient means to transform theory into best practices and promote problem-solving competencies. SGs can be developed to diagnose virtual patients that incorporate real data. The educational goals should include: (i) how to make the most out of the patient history and the physical examination by notably acquiring a good knowledge of pathophysiology, (ii) request tests/exams that are the most appropriate with the current differential diagnosis (iii) interpret the tests/exams results within a global patient view.

We describe diagnostic concepts that can be incorporated in serious games dedicated to medicine.

Diagnosis can be modelled as an iterated hypothetico-deductive loop (HDL): collect/elicit patient data, evaluate them, establish a differential diagnosis, repeat the HDL if needed. The initial steps of diagnosis consist of assessing the chief complaints and proceeding to a thorough history [2]. The differential diagnosis allows the physician to focalize his/her attention on specific body regions and anatomical systems during the physical examination leading to (perhaps) more specific hypotheses. When more than one hypothesis remains, diagnosis can be refined by requesting complementary exams leading to a new HDL. An IT approach to diagnosis can be built around the combination of expert heuristics[2], pathophysiology[3] and concepts burrowed from evidence-based medicine (EBM) [4][5], where decisions are based on weighted evidence. The generation of hypotheses during the initial steps is based on the prevalence of diseases that allow estimating prior probabilities. Post-test probabilities can then be established at the end of each HDL. Where appropriate,



"1. Visual inspection of a virtual patient (physical examination)"



"2. Initiating a blood film assessment (complementary exam)"

the choice of complementary exams will depend on two strategies: attempt to confirm the most probable hypotheses (given the current evidence) and attempt to refute potentially high-risk hypotheses. In short, this means selecting of exams that are specific (for confirmatory purpose) and/or sensitive (for refutation purpose). In Digital Pathology (DP), these considerations can help learners evaluate the cost of making false-positive and false-negative errors. For instance, a false-negative error in the context of a high-risk disease can be costly to the patient. Hence a global understanding of the patient case and a risk assessment of the competing hypotheses can help students in DP stratify the search of regions of interests (ROI) because they would know what image features they should attempt to confirm or refute first.

We are currently developing a technology platform dedicated to the realization of serious games in medicine. It incorporates generic reasoning modules that can combine expert heuristics, pathophysiology with concepts of EBM. Since annotated DP WSI can be integrated within the patient cases, such tools will contribute to illustrate (i) how applied sciences, medical devices and information technologies have become an integral part of modern medicine, and (ii) how each piece of information can contribute to an integrated approach to patient diagnosis and treatment.

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SEMI-AUTOMATIC QUANTIFICATION OF MRNA EXPRESSION IN WHOLE-SLIDE TISSUE IMAGES

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Introduction/ Background

For complex diseases, commercially available antibodies directed again specific protein sometimes lack the specificity required for clinical purpose. Therefore, novel techniques have been proposed recently to better assess the expression status of molecules at the mRNA level, e.g. by in situ hybridization. With such a technique, single-molecule signals can then be quantified on a cell-by-cell basis in whole tissue slides. However, highly heterogenous expression of mRNA are often observed at the tissue level, requiring the development of accurate and reproducible quantification methods.

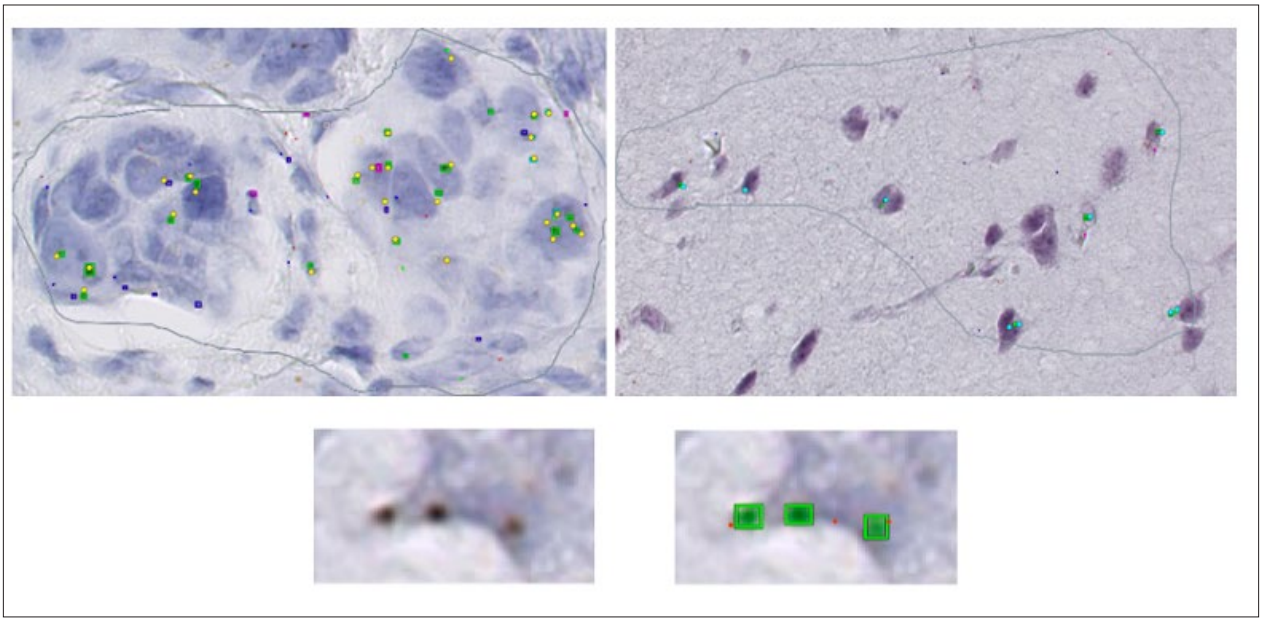
Aims

In this work, our aim is to propose and evaluate a methodology for the quantification of mRNA expression in digital slides to help biomedical researchers to analyze their large-scale imaging data. In particular, we will work on a dataset of breast cancer tissue samples and evaluate the accuracy of such a methodology to quantify Breast cancer susceptibility gene 1 (BRCA1) mRNA expression levels. BRCA1 is a tumor suppressor gene associated with the triple negative breast cancer (TNBC) subtype. An accurate technique to determine BRCA1 protein tumoral expression status in TNBC would allow for informed decision and choosing adapted treatments.

Methods

Data:

To design and evaluate our methodology, we use the data from [1]. In that study, the BRCA1 mRNA expression was assessed by in situ hybridization using RNAscope technology [2] (ACD-Bioke) for formalin-fixed, paraffin-embedded tissue samples. 88 glass slides were scanned (Hamamatsu, 40X, 0.23µm/pixel) and transferred on a Cytomine server (http://www.cytomine.be/) [3]. These slides correspond to 62 tumours (TNBC subtype). Using Cytomine web manual annotation tools, a total of more than 200 regions of interest were manually drawn by an expert in these whole-slide images. Then, independent assessment was performed by two observers in these ROI: they manually draw point annotations corresponding to tens of thousands of BRCA1 mRNA expression signals.



"Two Images with expert annotations and our system detection yellow point (left), cyan points (right) : expert annotations, in green area: the system detection Bottom: zoom showing that our detection is accurate in term of location"

Algorithms:

We have developed the Icytomine plugin to communicate between Icy and Cytomine. It allows the importation of the subtumor images at a desired resolution and the expert annotations from Cytomine and the exportation of detection results from Icy software (http://icy.bioimageanalysis.org) [5]]. In order to quantify mRNA signals, we combined several image processing routines in the Icy. Our workflow is composed of three steps: 1) color deconvolution [4] to extract the dark brown staining, 2) detection of spots based on undecimated wavelet transform [6], 3) post-processing to remove artifacts based on size, shape and color features. This transform is fast and ideal for representing isotropic objects. We will use a colocalisation coefficient to quantify the concordance between the experts. For a purpose of automation, we use scripts and protocols of Icy.

Results

An illustration of our results is given in *Figure*

Our ongoing large-scale empirical study stresses the need for more confident ground-truths hence more robust algorithms. Interestingly, our current strategy detects accurately spot locations. We believe it will help us to create a larger ground-truth dataset using expert proofreading. We will report at the conference our best quantitative results. Overall, we believe that our approach will speedup in a confident way the quantification of mRNA signals in whole-slide tissue samples.

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DIAGNOSIS OF THE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) USING A RAMAN-BASED SCANNER OPTIMIZED FOR BLOOD SMEAR ANALYSIS (M3S PROJECT)

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Introduction/ Background

In hematology, actual diagnosis of B chronic lymphocyte-leukemia (CLL) is based on the microscopic analysis of cell morphology from patient blood smear. However, new photonic technologies appear promising to facilitate and improve the early diagnosis, prognostic and monitoring of personalized therapy. The development of automated diagnostic approaches could assist clinicians in improving the efficiency and quality of health services, but also reduce medical costs.

Aims

The M3S project aims at improving the diagnosis and prognosis of the CLL pathology by developing a multimodal microscopy platform, including Raman spectrometry, dedicated to the automatic analysis of lymphocytes.

Methods

Blood smears were prepared on glass slides commonly used in pathology laboratories for microscopy. Two types of sample per patient were prepared: a conventional blood smear and a deposit of "pure" lymphocyte subtypes (i.e. normal B, CLL B, T and NK), sorted out in flow cytometry by using the negative double labelling technique. The second sample is used for the construction of a database of spectral markers specific of these different cell types. The preparations were analyzed with the multimodal machine which combines i) a Raman micro-spectrometer, equipped with a 532nm diode laser excitation source; ii) a microscope equipped with 40x and 150x lenses and a high precision xyz motorized stage for scanning the blood smear, and localizing x-y coordinates of representative series (~100 for each patient) of lymphocyte cells before registering three Raman spectra; these cells of interest being previously localized by an original method based on the morphology analysis. After the Raman acquisitions, the conventional blood smears were submitted to immuno-labelling using specific antibodies. For the establishment of the Raman classifiers, this post-acquisition treatment was used as reference to distinguish the different lymphocyte sub-populations. Raman data were then analyzed using chemometric processing and supervised statistical classifiers in order to construct a spectral library of markers highly specific of the lymphocyte type and status (normal or pathological).

Results

Currently, a total of 60 patients (CLL and healthy) were included in the study. Various classification methods such as LDA (Linear Discriminant Analysis), PLS-DA (Partial Least Square Discriminant Analysis), RF (Random Forest) and SVM (Support Vector Machine), were tested in the purpose to distinguish tumoral B lymphocytes from other cell types. These classification algorithms were combined with feature selection approaches. The best performances were around 70% of correct identification when a three-class model (B-CLL vs B-normal vs T and NK lymphocytes) was considered, and 80% in case of a two-class model (B-CLL vs B-normal lymphocytes). These encouraging results demonstrate the potential of Raman micro-spectroscopy coupled to supervised classification algorithms for leukemic cell classification. The approach can find interest more generally in the field of cyto-hematology. Further developments will concern the integration of additional modality such as Quantitative Phase Imaging on one hand to speed the exploration process of cells of interest to be probed, and on the other hand to extract additional characteristics likely to be informative for CLL diagnosis. In addition, the identification of prognostic markers will be investigated by confronting the photonic data to clinical patient information.

RAMAN SPECTROSCOPY-BASED CANCER DIAGNOSTIC PLATFORM FOR PATHOLOGY CLASSIFICATION IN BARRETT'S OESOPHAGUS AND ITS INTEGRATION INTO CLINIC

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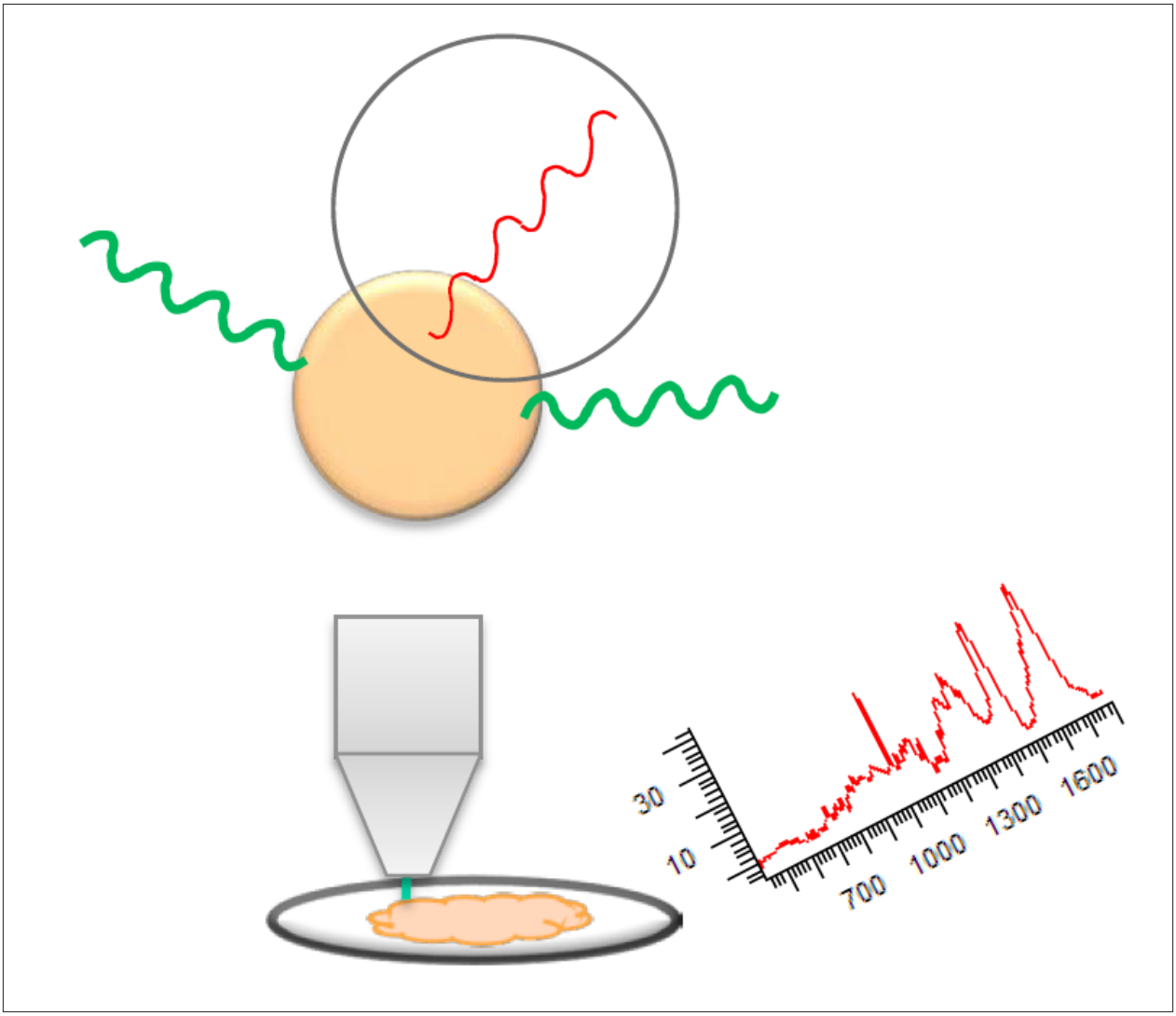
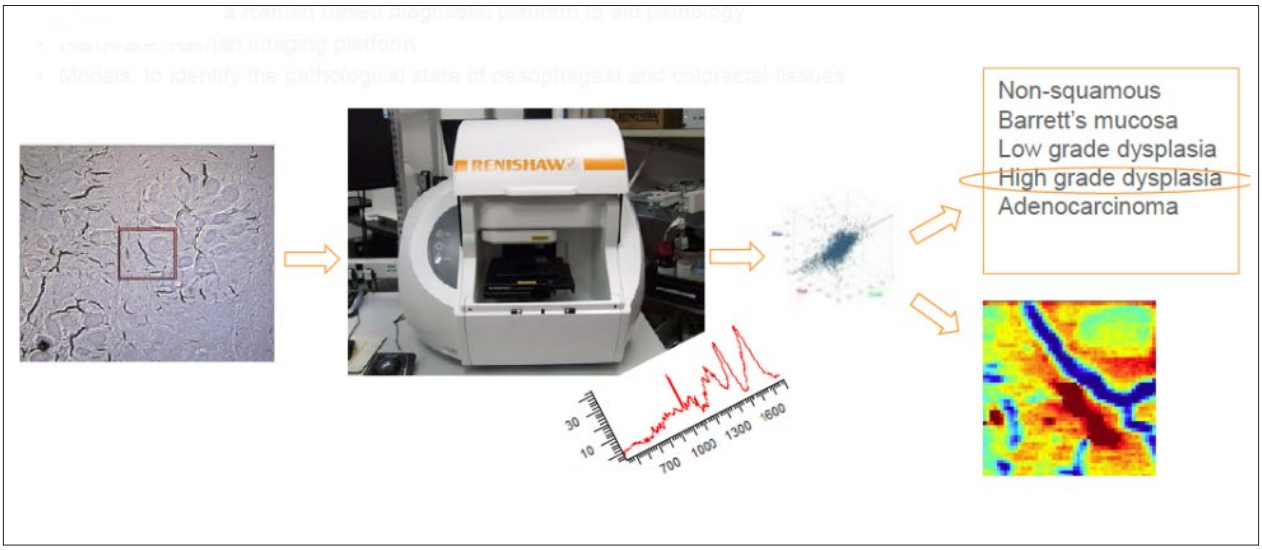
Introduction/ Background

Raman spectroscopy (RS) has been shown to accurately classify tissue pathology in a variety of conditions and organ systems. Much of this work has been performed using Raman microspectrometers on tissue sections.

Despite the demonstrated potential as an accurate cancer diagnostic tool, RS is yet to be adopted by the clinic for histopathology review. The Stratified Medicine through Advanced Raman Technologies (SMART) consortium has begun to address some of the hurdles (e.g. tissue sample



"Raman spectroscopy is based on inelastic light scattering and occurs when the biological sample is illuminated by monochromatic laser light. This provides a spectroscopic signature of all molecular constituents of the tissue sample."



"Raman spectroscopy can identify pathology states based on the chemical information, providing an objective highly specific and sensitive diagnostic method."

preparation, data collection, pre-processing and transferability) in its adoption for cancer diagnosis. SMART is a multicentre industry-clinical-academic collaboration with the aim of developing a pathology platform for advanced diagnosis, using developments in hardware and software. Renishaw's Streamline™ Raman imaging technology enables the collection of Raman spectra much faster without compromising signal to noise

Aims

This study aims to assess the ability of this technique to accurately classify tissue pathology, using an oesophageal tissue model. This demonstrates the project's mission to deliver a robust Raman based diagnostic platform to enable clinical researchers to stage cancer, define tumour margin, build cancer diagnostic models and discover novel disease bio markers.

Methods

Tissue was collected from the oesophagus in patients undergoing endoscopy or resection. Specimens were collected from patients with Barrett's oesophagus (BO), dysplasia and adenocarcinoma, and snap frozen in liquid nitrogen. 8µm tissue sections were placed onto calcium fluoride slides for spectroscopic measurement and with contiguous sections stained with haematoxylin and eosin (H&E) for histological comparison. Raman spectra were collected across homogeneous regions of tissue pathology, using Streamline™ acquisitions of 60 seconds/line, at 1.1µm spatial resolution. Classification models were constructed to discriminate pathology subtypes.

Results

Advanced multivariate statistical analysis tools were used to develop pathology classification models, which were then tested using leave-one-out cross-validation. Each sample was then classified using a 'voting classification' for all pixels from one sample. The sensitivity and specificity of this pathology classification model using RS to discriminate dysplasia/adenocarcinoma from BO produced sensitivity and specificities >80%.

By combining multivariate statistical analysis with Streamline™ Raman acquisition of spectral data, we have demonstrated good sensitivities and specificities. This study illustrates the potential of non-invasive rapid Raman spectral mapping measurements and development of a robust and validated oesophageal classification model that are able to classify tissue pathology, providing a diagnostic tool for researchers and clinicians with potential application to other pathology and tissue types.

RETAINED PTEN EXPRESSION PREFERENTIALLY IDENTIFIES MISMATCH REPAIR-PROFICIENT BREAST CANCERS

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Introduction/ Background

Loss of phosphatase and tensin homolog (PTEN) expression and alterations in mismatch repair (MMR) genes are regarded as early oncogenic events in breast cancer. It has recently been hypothesized that the polyadenosine tract in PTEN might be a target for mutation in MMR-deficient endometrial tumors. However, the frequency and significance of MMR alterations in breast cancer is debated, and their relationship with PTEN status has not been investigated in the breast.

Aims

In this study, we sought to explore the relationships between PTEN expression and MMR alterations and to define whether PTEN immunohistochemistry is a predictor of MMR status in breast cancer.

Methods

309 cases, including 261 invasive ductal carcinomas, no special type, 32 invasive lobular carcinomas, and 16 invasive ductal carcinomas, mixed types, carefully characterized from clinical and pathological standpoints, were reviewed and used to construct 11 tissue microarrays (TMAs). For each case, a mean of 4.5 tumor tissue cores (range 3 to 6 cores) was sampled, incorporating distinct topographic areas of the tumor, as well as matched non-neoplastic breast tissue, and, when present, associated in situ carcinoma. Taken together, 1381 spots were generated. Each TMA was subjected to immunohistochemical analysis of PTEN and the DNA MMR proteins MLH1, MSH2, MSH6 and PMS2. In order to allow a quick navigation within each TMA, and to minimize human-related biases, each stained slide was digitalized and blindly analyzed by two pathologist using a dedicated software able to segment TMA cores. The pattern of expression was therefore annotated manually on a digital database using a specific add-on module.

Results

According to clinicopathologic surrogate definition of intrinsic subtypes, PTEN protein loss was more frequent in luminal A-like and triple negative groups compared to luminal B-like carcinomas, as recently observed in other studies. MMR status in Luminal B-like tumors did not differ significantly between PTEN-retained and PTEN-loss groups, regardless HER2 amplification. In particular, retained PTEN expression was a predictor of MMR proficiency in approximately 35% of cases for this group. However, in luminal A-like and triple negative breast cancer groups, retained positive expression of MMR proteins was observed in 100% of cases showing PTEN wild-type immunohistochemical expression.

Discussion: The present study is the first to investigate PTEN protein loss in a large set of breast carcinomas based on DNA MMR status by immunohistochemistry. Our findings broaden the understanding of the biology underpinning breast cancer, suggesting that MMR alterations are likely to be independent of PTEN status in the majority of luminal B-like breast cancers and that, in a way akin to endometrial carcinoma, MMR deficiency could play a part in the development of PTEN alterations in luminal A-like and triple negative breast cancers. The integration of traditional pathology with cutting-edge digital tools allowed a rapid quantification of immunohistochemistry and effective data organization in this wide cohort multi-variable study.

Conclusion:

PTEN immunohistochemistry is a useful adjunct in the clinical evaluation of breast cancer patients, being able to capture all MMR-proficient luminal A-like and triple negative tumors.

COMPUTATIONAL TOPOLOGY BASED QUANTIFICATION OF HEPATOCYTES NUCLEI IN LIPOPOLYSACCHARIDE-INDUCED LIVER INJURY IN MICE

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Introduction/ Background

Automated high resolution scanning microscopes digitize large sets of histological samples and access the anatomical features of cells and tissues from the mm range down to a resolution of .25mpp (microns per pixel). The high quality of the scans allows for the collection of the quantitative morpho-topological features of cells and tissues from different samples, which can be coupled to functional information through, e.g., concomitant immunostaining. The basis for robust and accurate quantification of structural and functional features is the segmentation of regions of interest (ROIs) which define different elements within the scans. Due to acquisition artifacts and the diversity and variance of possible targets, the characterization and segmentation of ROIs in histological samples is difficult and challenging.

In recent years, computational algebraic topology, a field of mathematics, has established a robust and versatile way to obtain qualitative information from data. The most fundamental qualitative description of an object is given by the study of its topology, how the object is connected, how many holes it has, and of what type. That allows characterizing data sets according to their structure, increasing our understanding of their properties.

Aims

We propose a method for the robust segmentation of hepatocyte nuclei based on the principles of persistent homology, a tool of algebraic topology. We show the application of our technique in histopathological, whole slide images obtained from liver sections of lipopolysaccharide (LPS)-treated mice. The robustness is achieved by the introduction of persistent homology to characterize the hepatocyte nuclei. Its stability proves the usefulness of persistent homology; variations in the properties of the ROIs induce small changes in the resulting characterization. By means of this representation for the hepatocyte nuclei, the resulting segmentation is less sensitive to acquisition artifacts and natural variations of the images across batches of slides.

Methods

The sample space of this study consists of 856 cropped images of 616x616 pixels each, obtained from three specimens. Each image was fragmented into connected components at different scales. Persistent homology is used to study the inclusion relations between connected components. The outcome of such process is a persistence diagram that provides a low-dimensional projection of the image structure. From that representation, it is possible to use conventional statistical methods for segmenting hepatocyte nuclei. After the segmentation, we assess the performance in comparison to a gold standard segmentation validated by experts.

Results

The computational topology approach proposed successfully detected hepatocyte cells under several natural variations. We evaluated on a per-pixel basis how the segmentation performs on: i) all nuclei in the images, ii) big round nuclei considered belonging to hepatocytes cells (accuracy 87.2%, recall 80.3%), and iii) nuclei regarded to non-parenchymal cells.

A GRAPH-BASED DIGITAL PATHOLOGY APPROACH TO DESCRIBE LYMPHOCYTE CLUSTERING PATTERNS AFTER RENAL TRANSPLANTATION

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Introduction/ Background

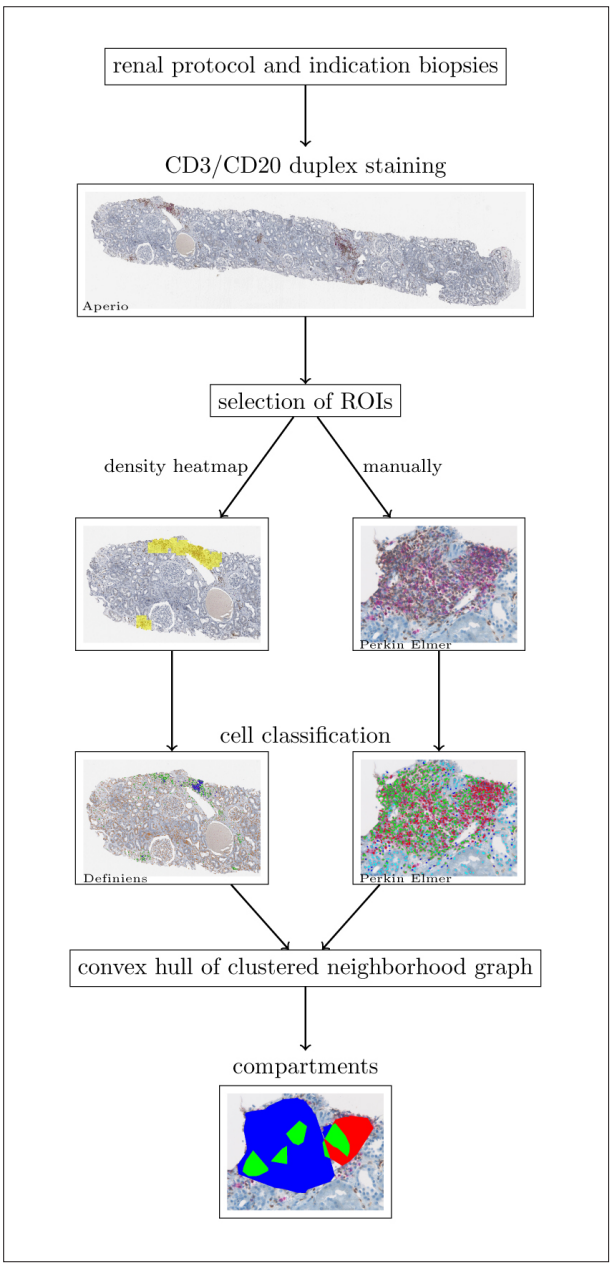
Renal transplantation (rTx) induces an adaptive immune response against foreign donor antigens mediated by lymphocytes of the recipient. Local accumulation of B- and T-cells is an important component of this response enabling and controlling immune cell interactions [1]. Combining digital microscopic images with network analysis [2][3] opens new perspectives to study the spatial dimension of lymphocyte clustering and to model their potential interactions.

Aims

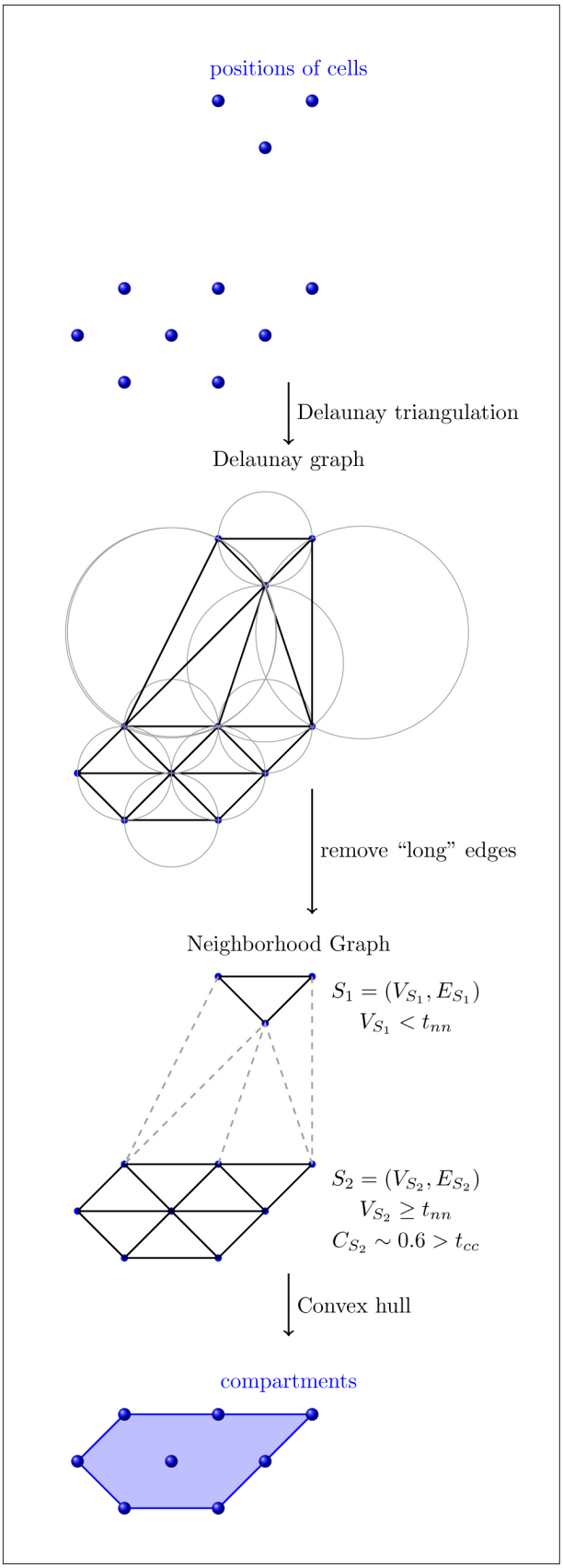
The aim of this study is to characterize the range of B- and T-lymphocytic infiltrates below the threshold of rejection defined by the Banff classification [4][5] and to propose a mathematical description of immune cell clustering for use in systems medicine approaches.

Methods

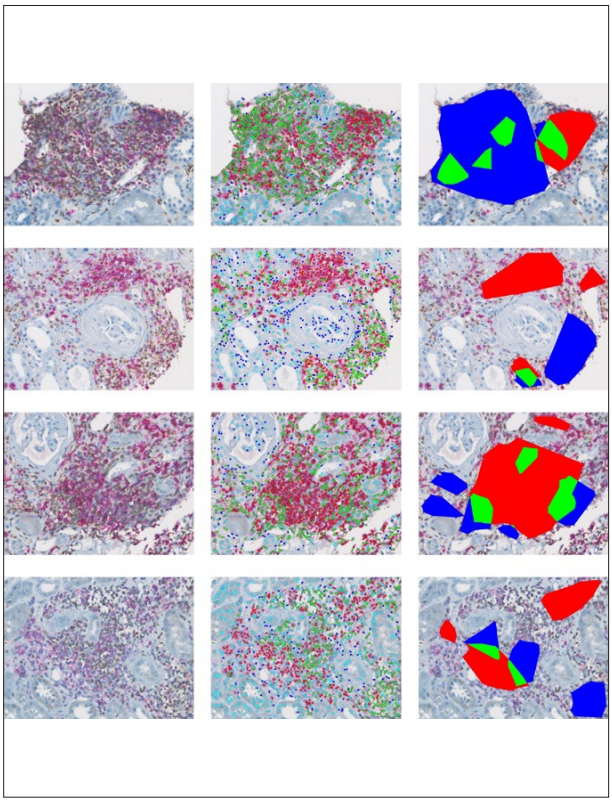
We established a workflow to comprehensively characterize lymphocyte clusters and compare their morphological features with organized structures such as secondary or tertiary lymphoid organs (TLO/SLO) [6]. 51 renal protocol and indication biopsies from 13 patients without evidence for severe rejection over 10 years were stained by CD3/CD20 duplex immunohistochemistry. Whole slide images (WSIs) were acquired to automatically detect biologically relevant regions of interest (ROIs) by means of density maps for lymphocytes (image analysis workflow illustrated in Fig. 1a). They are generated from single nuclei identification using an auto-adaptive random forest pixelwise classifier ("nucleus container" module [7], Definiens, Germany). We implemented a graph-based tool in Java using individual cell coordinates to identify cell compartments (Fig. 1b) and applied it to each selected ROI. For this, a neighborhood graph is built by Delaunay triangulation and Euclidean distances. This analysis allows describing their specific clustering behavior based on features as described in [8]. The convex hull of the neighborhood graph allows a visualization of B- and T-cell compartments.



"Fig. 1a: Flow chart of image analysis.



"Fig. 1b: Cluster description by connected components (S1, S2) of a neighborhood graph. Dashed lines illustrate large Euclidean distances. In this example, S2 fullfils threshold for clustering coefficient C."

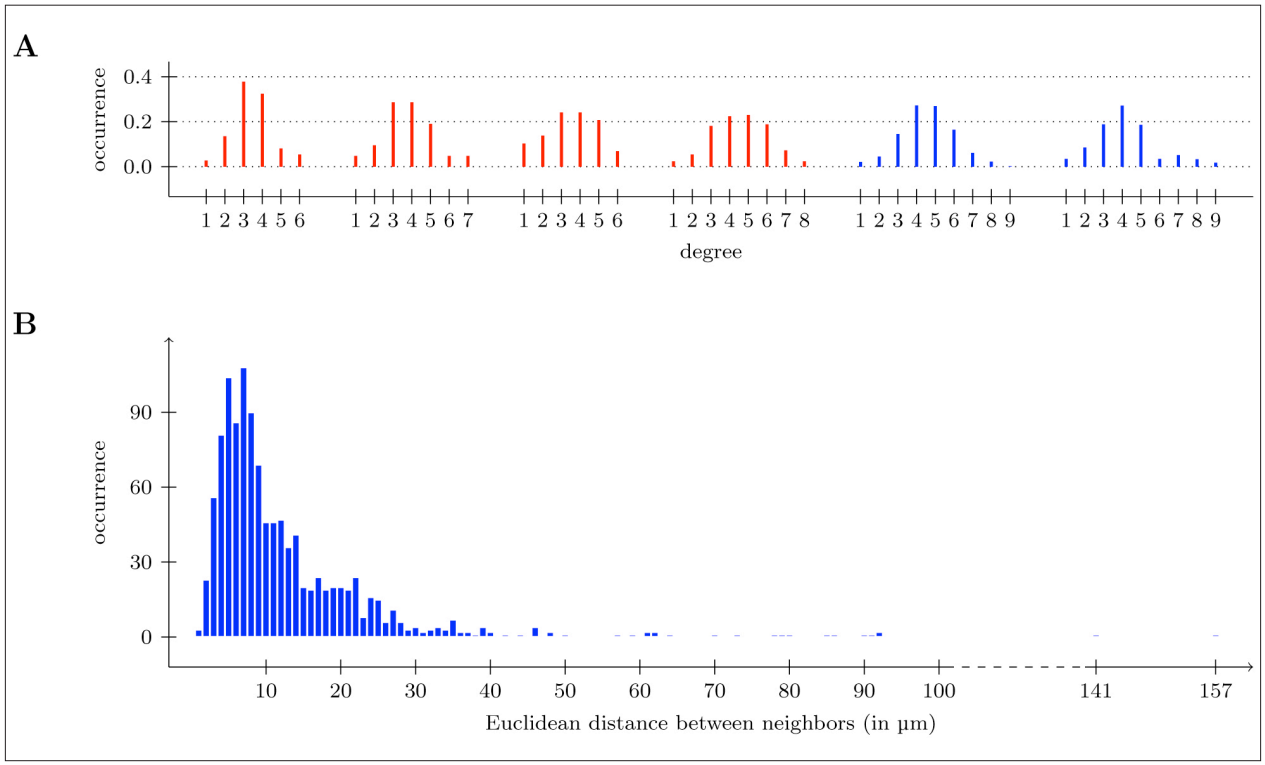


"Fig. 2: Left: Regions of interest. Middle: Cell classification (red: B-cells, green: T-cells, blue: other cell types). Right: Detected compartments (red: B-cell clusters, blue: T-cell clusters, green: intersections)."

Results

We identified B-cell rich compartments in about 55% of 150 ROIs in kidney tissue after successful transplantation (examples in Fig. 2). The B-cell compartments in rTx tended towards smaller overall size with on average about 90 cells in a B-cell cluster compared to more than 600 B-cells observed in mature TLOs and SLOs and they showed less prominent spatial organization (average degree on average 3.92 instead of 4.97; degree shows generally Poisson distribution as illustrated in Fig. 3A). Further, the graph analysis confirmed lower B-cell density (Fig. 3B displays the exponential character of the spatial B-cell distribution in a selected ROI), a different ratio between T- and B-cell compartments, and more frequent overlap between both regions than in mature lymphoid structures.

We conclude that the graph-based approach is feasible to distinguish relevant immune cell patterns in rTx and provides a useful mathematical description of neighborhood relationships between immune cells and their spatial organization. The workflow has the potential to improve throughput and robustness of immune cell evaluation for use in translational science.



"Fig. 3: A: degree distribution of an example neighborhood graph (Fig. 2 top), red: B-cell components, blue: T-cell components. B: Histogram of B-cell distribution, i.e., Euclidean distance between neighbors in Delaunay graph."

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GRAPH-BASED APPROACH FOR SPATIAL HETEROGENEITY ANALYSIS IN TUMOR MICROENVIRONMENT

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Introduction/ Background

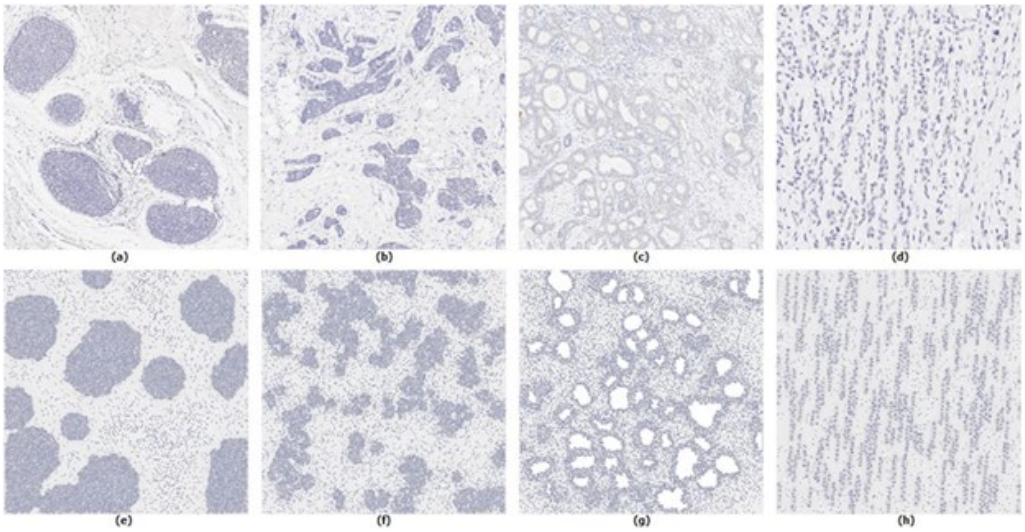
The interaction between tumor and surrounding microenvironment (TME) is recognized as playing an important role in the progression of the disease. Understanding of the interaction between tumor and immune system is the focus of several studies dedicated to the improvement of cancer immunotherapy effectiveness[1]. On the other hand, it has been shown that invasion and metastasis of breast tumors is influenced by collagen organization at the tumor-stromal interface[2]. The characterization of such interactions relies on an efficient spatial distribution quantification of TME. Graph-based analysis tools are the best suitable to answer this question as they have the ability to represent spatial arrangements and neighborhood relationships of different tissue components[3].

Aims

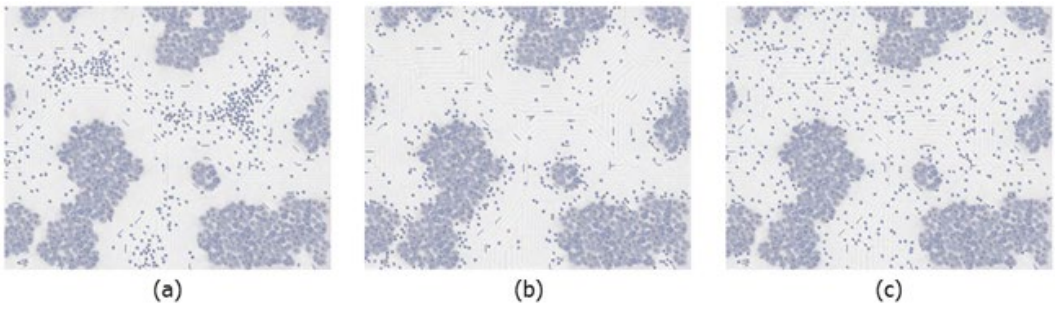
In this work, we propose a novel approach to characterize the spatial relationships between cancer cells and TME components in breast tumors, using graph theory and sparse sets' mathematical morphology (MM). The tools of morphology on graphs were first used in[4] to study the neighborhood relationships between cells in germinal centers from lymph nodes, then in[5] for semantic spatial configuration modeling in histopathology. In our study, we propose new morphological descriptors characterizing the tumor architecture and the interactions with TME cells.

Methods

Towards a better evaluation and understanding, we use simulated data of different breast tumor types (fig.1, fig.2), where locations of cancer nuclei (CN), fibroblasts (synthesizers of collagen, FN), and lymphocytes (LN) are already known. In order to set neighborhood relationships between different cells, Delaunay graph[3] is first reconstructed on all cells, and alpha-shape filter[5]

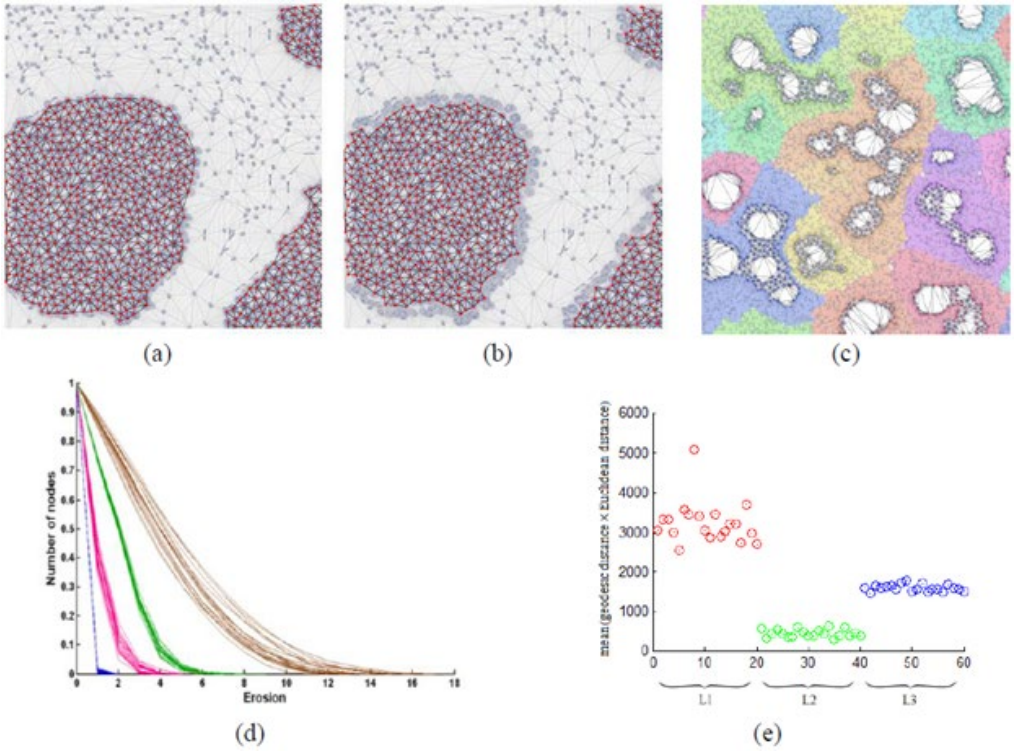


"First row real data, second row simulated data. (a-e) Ductal Carcinoma In Situ, DCIS. (b-f) Invasive Ductal Carcinoma, IDC. (c-g) Tubular Carcinoma, TC. (d-h) Invasive Lobular Carcinoma, ILC (showing Indian file architecture)"



"Simulated data of an IDC with lymphocytes in different spatial distributions. (a) Lymphocytes stood away from tumor aggregates. (b) Lymphocytes are surrounding the borders of tumor aggregates. (c) Lymphocytes are uniformly distributed."

is applied to circumvent border effects, giving new graph denoted G (fig.3.a). The designed features are extracted basically from two different morphological operations. The first operation is composed of successive morphological erosions[4] applied to the subgraph induced by CN (denoted SGC, fig.3.a), repeated until the subgraph is null. The curve given by the number of CN in terms of erosions provides 3 significant characteristics



"(a) SGC = (red nodes, black edges). (b) First erosion of SGC. (c) Variation of the number of CN in terms of erosion. (d) Labeled-dilation with non-overlapping control. (e) Mean distance of LN from TAs in the 3 different configurations shown in fig.2."

(fig.3.d). i) The origin slope describes the number of CN on the boundary of tumor aggregates (TA) and, thus, the tumor-stromal interface (fig.3.b), ii) the area under curve (AUC) reflects the density within TAs, and iii) the number of iterations outlines the morphologic radius of the largest TA and, consequently, the geodesic distance of the farthest tumor cell from LN and/or FN. The second morphological operation is composed of successive morphological dilations applied to SGC with non-overlapping control of labeled connected-components (fig.3.c). The goal behind this operation is to investigate the TME cells surrounding each TA. The ratio between the number of LN and the number of CN, and the means of the Euclidean and the geodesic distances of LN from CN on the boundary are calculated for each TA (fig.3.e).

Results

In this work, we have briefly presented a conceptual framework for analyzing the architecture of breast tumors and the interactions with the surrounding microenvironment. New graph-based features were proposed to characterize the spatial distribution of TME components and were tested on simulated data. In our future works, we will include adipose tissue[6], blood vessels and endothelial cells. We will also focus on the anisotropic characterization of collagen, and test the approach on real dataset.

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FRACTAL BEHAVIOR OF GLEASON AND SRIGLEY GRADING SYSTEMS

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Introduction/ Background

Prostate cancer remains one of the major malignancies of modern society. The need of grading this malignancy is still in dispute. Two major grading systems have emerged and are world-wide adapted: Gleason grading system [1] and Srigley grading system [2]. Both systems use optical subjective descriptions of different architectural growth patterns of prostate adenocarcinoma. The fractal dimension (FD) is used in the medical field as an objective feature for describing a given image rather than showing a precise value for a known fractal. The FD can be an objective measurement for different patterns description.

Aims

The aim of our study is to assess the fractal behavior of images labeled according to Gleason and Srigley grading systems both in terms of in-class and inter-class variation.

Methods

299 Gömöri stained microscopic digital images of prostate adenocarcinoma were labeled independently according to Gleason and Srigley patterns. Each image was firstly transformed to grayscale then a maximum cropped square of the image was resized to a standard 256x256 pixel image. For the resulted images the fractal dimension was approximated with two different algorithms: a standard box-counting algorithm (applied to the binary image obtained with Roberts's method for edge detection) and a novel algorithm that is applied to the grayscale version of the image consisting in the ratio between image's volume and area (R-VA) at different scales [3]. In-class variation was assessed as the average standard deviation (SD). Lower SD means better discrimination. For the inter-class variation assessment each class was compared with all other classes using a two-tail, Student's t-test. The resulted value was defined as the ratio between the statistically different means and the total number of comparisons. The maximum possible value for Gleason grading system was 28, because there were no images labeled as Gleason pattern 1, while for the Srigley grading system the maximum possible value was 6.

Results

In-class variation was 0.045 using the box-counting algorithm and 0.048 using the R-VA algorithm for Gleason grading system and 0.161 using the box-counting algorithm and 0.178 using the R-VA algorithm for Srigley grading system. Inter-class variation was, for Gleason grading system 13/28 using the box-counting algorithm and 20/28 using the R-VA algorithm while for the Srigley grading system was 3/6 using the box-counting algorithm and 5/6 using the R-VA algorithm respectively. Srigley grading system seems to perform better than Gleason's on inter-class variation, but has lower performance on in-class variation. Nevertheless, we must note that there is a large difference between the two systems regarding the number of classes. The FD computed with the R-VA algorithm has better discrimination results than the one computed with the box-counting algorithm in both grading systems, thus proving once again the R-VA's performance [3].

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CLASSIFICATION OF DEGREE OF DIFFERENTIATION OF COLORECTAL NEOPLASM BY CHANGES IN THE BETTI NUMBER

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Introduction/ Background

The diagnosis of pathology provides important information for determining the treatment protocol, but other than in developed countries, the number of pathologists is not sufficient. Even where the number is sufficient, they are often unevenly distributed, with most pathologists in large hospitals in metropolitan areas, and adequate services cannot be provided to all patients. For this reason, it is desirable to develop ways to allow for remote diagnosis or to develop automatic diagnostic systems. Digital diagnostic systems based on pattern-recognition techniques have been developed. Because the morphology of cancer tissue is quite complex, a library of all types of cancer morphology would be huge, it would require a high-performance computer system, and the costs in money and time would be very high. This makes it difficult to create an effective system.

Aims

Recently, a new method based on the homology theory for analyzing histological digital images has been developed. The method evaluates the Betti numbers in a unit area of an image of a colon to determine the region of interest (ROI). The Betti number is an important index for homology theory, and can be used to assess the degree of connectivity in tissue. This method, however, cannot distinguish between different types of tissue. When a tumor forms in the colon, because of the excessive growth of nuclei, the connections increase and become tight. The absolute value of b1 (the one dimensional Betti number) will be high, and the ratio of change will be small. When a tumor becomes more poorly differentiated, it frequently happens that the ties between the cells are weakened, and the intercellular areas are filled with impurities. Because of these impurities, the absolute value of b1 is very high. Its influence, however, will disappear immediately. Namely, b1 decreases very quickly, when we decrease the binarizing threshold. We calculate the decay ratio of the betti number, by changing the binarized thresholds. Our aim in this talk is that using this information, we will classify the degree of differentiation of colorectal neoplasm.

Methods

In the colon, cancerous regions show a stronger color intensity when stained with hematoxylin. This stains blue, and so the distribution of blue in an image indicates the cancerous areas. Thus, for each image, we measured the peak of the blue distribution, and we will refer to it as the reference value. To determine the binarizing threshold, we multiplied the reference value by 0.55 to 0.72, in increments of 0.01. The binarization was carried out in gray scale using these values. We call this interval the reference interval. We may assume that there was no pathological information outside of the reference interval.

Results

The calculated results can be approximated by quadratic functions. The distribution of the coefficient on the squared term and the x-coordinates of the vertices are shown. We can see a characteristic distribution for each type of cancerous tissue.

As the binarizing threshold decreases, the images gradually fade to white, and the structure of the tissue is lost. Under the proposed procedure, in areas where the connections in the tissue are tight and clear, b1 changes slowly. Conversely, where the connections are vague, such as in a background area filled with impurities, it changes very quickly. The state of this change can be considered an expression of the strength of the connectivity, and it differs by type of cancerous tissue.

DEEP CONVOLUTIONAL NEURAL NETWORKS FOR HISTOLOGICAL IMAGE ANALYSIS IN GASTRIC CARCINOMA WHOLE SLIDE IMAGES

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Introduction/ Background

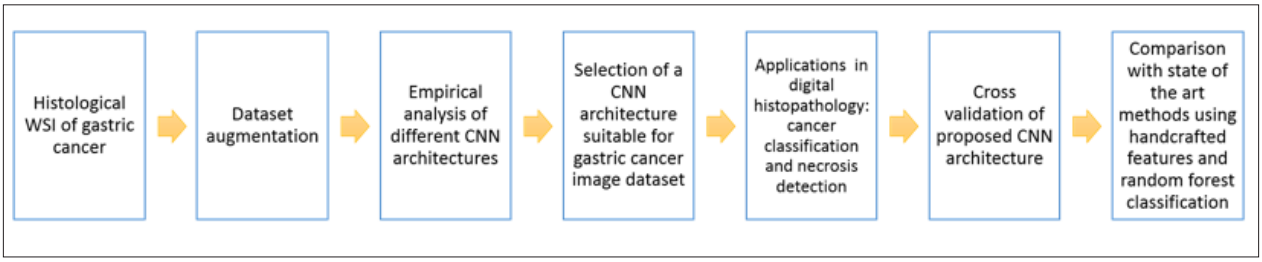
In this paper, histopathological whole slide images of gastric carcinoma are analyzed using deep learning methods. A convolutional neural network architecture is proposed for two classification applications in H&E stained tissue images, namely, cancer classification based on immunohistochemistry (IHC) into classes Her2/neu+ tumor, Her2/neu- tumor and non-tumor, and necrosis detection based on existence of necrosis into classes necrotic and non-necrotic. The studies in [1] and [2] explored computer-aided classification using graph-based methods and necrosis detection by textural approach respectively, which are extended using deep convolutional neural networks. Performance is quantitatively compared with established handcrafted image features, namely Haralick GLCM, Gabor filter-banks, LBP histograms, Gray histograms, RGB histograms and HSV histograms followed by classification by random forests, another well-known machine learning algorithm.

Aims

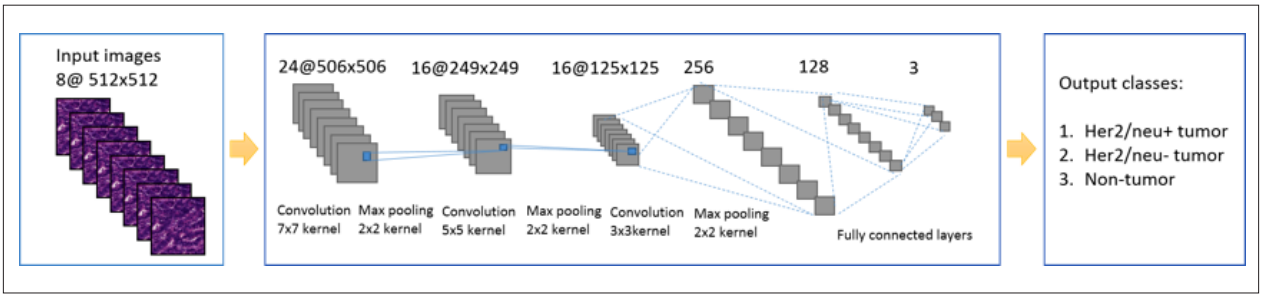
Convolutional neural networks (CNN) have recently gained tremendous attention in general image analysis [3]-[5]. There has also been an emergence of deep learning in digital histopathology for diverse classification and detection problems [6]-[8]. The prime motivation behind this work is that no previous study has explored deep learning for the specified goals in gastric cancer WSI. Automated cancer classification can assist pathologists in computer-aided diagnosis in H&E stained WSI without the requirement of IHC staining, thereby reducing preparation and inspection times, and decreasing inter- and intra-observer variability. Necrosis detection can play an important role in prognosis, as larger necrotic areas indicate a smaller chance of survival and vice-versa. Moreover, most deep learning studies have used smaller image sizes mainly due to memory restrictions of GPU, however, we consider larger regions in order to preserve context i.e. neighborhood information and tissue architecture at higher magnification. Further, this method is independent of nuclei segmentation, hence its performance is not limited by segmentation performance as in [1] (evaluation details in [9]).

Methods

Firstly, standard data augmentation techniques are applied on the available gastric cancer WSI dataset and thousands of images of size 512x512 are generated. Different CNN architectures are empirically studied to observe the behavior of variation in model characteristics (network depth, layer properties, training parameters, etc.) by training them from scratch on a representative subset of whole data for cancer classification. One of these is the Imagenet model [4], however it doesn't perform desirably on the representative dataset. The self-designed CNN architecture with best classification rates is selected. Later, the proposed CNN is also applied for necrosis detection. Performance is compared with state of the art methods using handcrafted features and random forests. For evaluation, randomized three-fold stratified shuffle split and leave-one-patient-out cross validations are used.



"Fig.1. Schematic overview"



"Fig.2: Proposed CNN architecture"

Results

Conclusion:

A self-designed CNN architecture is proposed for image analysis (cancer classification based on IHC and necrosis detection) in H&E stained WSI of gastric cancer. Quantitative evaluation shows that deep learning methods mostly compare favorably to state of the art methods, especially for necrosis detection. In future the aim is to expand the current WSI dataset and to improve the CNN architecture for optimal performance.

"Fig.3. Average cross validation accuracy for (a) cancer classification (b) necrosis detection"

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