11th European Congress on Telepathology *and* 5th International Congress on Virtual Microscopy

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CONGRESS ABSTRACTS

Editors: Vincenzo Della Mea and Roberto Mencarelli

International Academy of Digital Pathology
International Academy of Telepathology
Società Italiana di Anatomia Patologica e Citopatologia diagnostica

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Congress Abstracts

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[Please note that selected short papers from the congress will be soon published as a supplement of the journal Diagnostic Pathology, www.diagnosticpathology.org - cite them if you need]

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SCIENTIFIC ADVISORY BOARD

2012 – The beginning of a new world for Digital Pathology

Vincenzo Della Mea and Roberto Mencarelli
Congress Chairmen

The series of the biennial European congresses on Telepathology is reaching with this Venice edition its 11th appointment: more than 20 years of research in a continously growing field. It is also the fifth time the congress brings the additional name of International Congress of Virtual Microscopy: eight years ago the International Academy of Telepathology decided to recognize this way the importance of virtual microscopy, also called Whole Slide Imaging, in the renovation of the telepathology scenario. In the last congress the International Academy of Digital Pathology was also founded, to replace and continue the great work of the International Academy of Telepathology that organized most of this congress series. This step recollects under the single term "digital pathology" all the technologies that are tranforming the traditional, "analog" pathologist in a digital professional that work on digitized slides, with software tools that enable to better exploit his/her knowledge, leaving routine details to the computers and networks. Once slides are digitized, a whole bunch of applications come natural and available: telediagnosis, teleconsultation, e-learning, long term storage, up to image analysis, with the forthcoming field of digital immunohistochemistry. No need to carry out additional operations: slides are ready for further digital treatment, present and future.

Digital Pathology is made possible not only thanks to the research grown in the last many years and presented at every Congress of this series. Every year the processors power increases, memory becomes larger and larger, and thus computers become more and more adequate to such large, information-filled objects that are the so-called digital slides.

This 2012 Congress hosts more than 80 presentations from 23 countries of the world. It represents the current state of the art in the field of digital pathology: what can be heard and seen here will represent the future of Pathology in the short, mid and even long term, thus providing insights on a new world which the traditional and crucial work of the pathologist should remain under his/her own control, made easier and more productive through digital tools. Thank you for being here.

SCIENTIFIC SESSIONS

A1: TELEPATHOLOGY

Fifth Generation Telepathology Systems: The Whole Slide Image-Enhanced Dynamic Robotic Telepathology System

Braunhut BL, Graham AR, Richter LC, Webster PD, Krupinski EA, Bhattacharyya AK, Weinstein RS

USA

Background: Telepathology is the practice of pathology over distances using video-imaging equipment and a telecommunications network. Two workflow paradigms for telepathology practice are a subspecialty pathology practice (SPP) model and a case triage practice (CTP) model. With the CTP model, developed at the University of Arizona, the telepathologist on call can render a diagnosis independently or, regardless of its subspecialty category, has the option of referring the case to a subspecialty pathologist as needed.
Findings: Surgical pathology teleconsultations providing real-time quality assurance (QA) services were established between the University Medical Center (UMC) in Tucson, Arizona, and the Havasu Regional Medical Center (HRMC) in rural Lake Havasu City, Arizona, 300 miles away. HRMC had a single on-site pathologist. From 2005 to 2009, 1815 cases were reviewed by one of ten UMC case triage telepathologists. Each faculty pathologist had an area of surgical subspecialty expertise. 90.9% of cases were signed out directly by the on-service triage pathologist, without consultation with a subspecialty pathologist. The majority of cases were outside of the triage pathologists areas of subspecialty expertise. The diagnostic concordance of the telepathologist's diagnosis with the original pathologist's diagnosis was 94.3%. Major and minor discrepancies were 2.90% and 2.83% respectively. The discordant cases were re-reviewed by light microscopy at the UMC daily QA conference.
Conclusions: This study supports the use of the CTP workflow model for a telepathology QA service. Subspecialty surgical pathologists can efficiently and accurately render QA second opinions outside their areas of subspecialty expertise by telepathology. This workflow model will be applicable to practices using fifth generation telepathology systems.

A2: TELEPATHOLOGY NETWORKS

The Eastern Quebec telepathology network : a support to the improvement of the public health care system

Bernard Tetu, Marie-Pierre Gagnon, Genevieve Roch , Jean-Paul Fortin
Canada

Introduction: The Eastern Québec Telepathology Network is aimed at providing uniform diagnostic telepathology services in a territory of 408,760 km2 with a population of 1.7 millions inhabitants and which density, in certain areas, is as low as 0.4 inhabitant/km2. The network has been designed to avoid surgeons in smaller community hospitals to postpone surgeries requiring a frozen-section or to transfer patients to regional hospitals because of the lack of stable pathology coverage. It also allows pathologists who work alone to rapidly obtain a second opinion from a colleague. The project was initiated through funding of both the Quebec Ministry of Health and Canada Health Infoway as part of the Quebec quality assurance program. This study is aimed at providing an interim evaluation of the benefits of this network on the health care organization.
Methods: The project has been implemented in 21 sites, each equipped with a whole slide scanner, a macroscopy station, a videoconferencing device and a viewer / case management and collaboration solution. Of the 21 sites, 6 are devoid of a pathology laboratory, two have no pathologist and 5 have only one pathologist on site. Statistics on the number of slides scanned and the turn-around time for each step of the diagnostic process are obtained regularly. Results: Since the beginning of the implementation in 2010, 1 639 slides were scanned for primary diagnosis, 248 for second opinions from pathologist to pathologist, 377 for frozen sections and 81 for immunohistochemistry. Telepathology also allowed the transmission of gross images for diagnosis. One hundred twenty-five (125) sessions of videoconference allowed pathologists to supervise either the selection of a specimen for frozen section or the gross description by a technician and 569 gross images were taken for diagnosis. The turn-around time of frozen sections went down from 26,7 minutes in the first tests to 16,3 minutes in the fall of 2011. The major benefits since obtained so far with the network are: 1) expected interruptions of the frozen section coverage were avoided in two hospitals and local surgical and pathology technical activities were maintained; 2) the merging of smaller laboratories into a sub-region was encouraged with the result of a more stable frozen section coverage and an attractive effect on the recruitment of young pathologists; 3) the videoconferencing and grossing station allowed for real-time communication between a pathologist and the remote technician for grossing of specimens without having to send specimens over; 4) frozen sections were provided to oncologic surgeons in hospitals devoid of pathology laboratory; 5) many expert opinions were obtained with a significant reduction of the turn-around time; 6) surgeons obtained overnight diagnoses of urgent biopsies, despite the lack of an on-site pathologist; 7) several technical procedures were standardized (staining, sectioning, reporting).
Discussion and conclusion: The Eastern Québec Telepathology Network has been designed to improve overall pathology coverage in this region. In a short period of time, an improvement of medical cares to patients is already apparent.

Development of a nationwide telepathology quality control program for cancer diagnosis in China

Zhongjiu Zhang, Guangming Gao, Je Chen, Chao Hui Lu, Chen Zhou
Canada, China

Background: In order to improve the quality of pathology diagnosis of cancer, the Ministry of Health of China facilitated and developed a nation-wide telepathology consultation and quality control program in 2011.
Designs: An internet based telepathology platform (www.mpathology.cn) was built as the hub for the program, which connected hospitals and expert consultants. 80 expert consultants were selected from 356 pathologists with provincial and national reputation. 60 hospitals were selected from 87 volunteer hospitals in 17 provinces to participate the phase I of the program.
Results: The program consists of two parts: telepathology consultation and quality control. Each participating hospital is required to send WSI of at least 300 cases annually for a second opinion. The participating hospitals are required to submit WSI of every cancer diagnosed for quality control, which is carried out by expert consultants every 3 month on 10% randomly selected cases. The accuracy of cancer diagnosis and quality of tissue section are to be reviewed, assessed, and submitted to provincial and national pathology quality control centers.

Conclusion: A nation-wide telepathology program supported by government would play a pivotal role in promoting the use of telepathology for consultation and quality control in cancer diagnosis in China.

The Open European Nephrology Science Center as a Open Science Center - a platform for scientific data and image management

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Germany

The Open European Nephrology Science Center (OpEN.SC) is a repository for research related clinical data supported by the German Research Foundation. This project started as an Science Center related to the medical domain of nephrology. During the complete developmental process of this project the processes and database structures were designed and implemented independently from any medical domain. The main idea is to allow a connection of data of various medical domains for pattern analysis and study recruitment as a Open Science Center.
The platform supports the reuse of clinical for translational medicine as a cornerstone for the further scientific development in Medicine. It consists of process oriented tools for data import, management, analysis and presentation and solved the two main problems in scientific data and image management: storage of data and images taking to consideration the legal issues (secure patient data) and save the intellectual properties of the diagnostic and therapeutic process by the physicians.
All processes of scientific data and image management were modeled as business processes using BPMN (Business Process Modelling Notation by OMG). Process models allows to orchestrate and synchronize web services in a Service Oriented Architecture. At the backend an Apache Geronimo-Application Server ensures the availability and executable of the web serices. The Open Source database engine PostgreSQL is used to store the data. The user interface is realized by OpenSource Liferay Portal. Different tools for retrieval and image analysis are available.
The Open European Nephrology Science Center is a flexible and scalable platform for scientific data and image management. The process oriented modelling offers the opportunity to adapt the system for each specific use case and any type of data management model. The process models can be used for business simulation to evaluate the impact of changes very early.
The management of clinical data for research purpose is quite important especially in relation to tissue bio banks. The storage of information, material, media of different types such as Whole Slide Images and tissue will be the clue for the availability of clinical data for pattern analysis and study recruitment. Scientific data management reflects the secondary use of very valuable clinical data and should cover different aspects of data handling: data import, storage, retrieval, access and presentation concerning all legal issues and changing as well as increasing requirements to storage, retrieval and analysis.

Modern communication in medical science using an internet based project management system and file exchange platform

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Germany

Background: Modern communication is largely based on information exchange via email and mobile telephone lines. Networking - nationally, internationally or even within one institution needs fast and reliable communication with instant access not only to specific scientific contents but also to progress-tracking of each project. We therefore implemented an internet based information platform using modern project management software.
Methods: As our scientific group consists not only of researchers employed by the Institute of Pathology but also of medical students scheduling their research efforts along with their study time table we implemented an internet-based information system using the commercially available project management software package eGroupWare. Projects and sub-projects for each part of our studies were created with time frames and duty responsibilities. Each member of our research group is given access by individual logins as well as specific rights and privileges. Work progress is to be recorded and updated by each group member to monitor and focus manpower and scientific workload. To exchange data, especially literature a shared folder using the dropbox.com platform was opened to every group member.
Results: Due to web based installation tools offered by the hosting service installation of the web interface was facilitated and the web front end could be published within 24 hours. Creation of user accounts and user groups as well as of each project with sub-projects needed justifiable practice with eGroupWare, which also provides an online help system. Regular meetings as well as appointments for coordinated research are scheduled using the included calendar tool. Research progress is recorded and can be visualized by gant charts. Exchange of data

files, which do not include classified data (e. g. patient information), is easily accessible by the common dropbox folder.
Conclusion: Modern internet based tools including project management software facilitates and accelerates communication in medical science and allows fast tracking of work progress. The use of internet based exchange platforms will therefore also help in national and international science networks.

Webconference mixed with virtual slides as a pedagogical tool to improve pathology practice in the French Midi-Pyrenees area.

Marie-Laure Quintyn-Ranty, Celine Guilbeau-Frugier, Michel Jacob, Philippe Gil, Emmanuelle Uro-Coste, Yvan Nicaise, Marie-Bernadette Delisle
France

Midi-Pyrenees is the second largest region in France and has a low population density (63 inhabitants/km2). Consequently, meetings are often challenging, particularly in winter. Pathology is a part of medicine, which diagnoses tissular lesions on slides, based only on visual expertise. Moreover, pathologists are less numerous and predictive data shows that their population will be shared by 50% in 2020. Anyway, complexity of histopathology diagnosis requires a mandatory regular control and upgrading of professional knowledge.
Training is long and demanding. The aim is to accomplish diagnosis training.
We imagine a friendly environment to enable us to share virtual slides with medical students or senior pathologists.
Slides were scanned on « NDP Nanozoomer » Hamamatsu, in Rangueil University Hospital, in Toulouse. The virtual slides were loaded in to a computer server, with an external hard disk. The access wass secured by a code. The development internet portal and of the multimedia accommodations were provided by the Paul Sabatier University TIC/TICE and multimedia service, in collaboration with Hamamatsu that provides the program patch with the NDP viewer.
Thus it was possible:
- for any student or pathologist to connect to the server to see the didactic observations. The students could see the slides as often as they wished.
- to organize virtual meetings from personal computers with microphone and web cam. Sound and images were travelling by web. The virtual slides could be observed by everybody, with the possibility for every one to speak, to remote on the virtual slide to show a particular area. A moderator gave the right to speak or to move on the slide.
Everyone could see the same area of the slide at the same time. It was possible to take memos at the same time in a specific area and to save them on the computer or send them by mail.
This tool is powerful for pathologist e-learning by using a web site associating webconference and virtual slide consultation.

B1: COMPUTER-AIDED DIAGNOSIS

Out-of-sample extension of diffusion maps in a computer aided diagnosis system. Application to breast cancer virtual slide images.

Philippe Belhomme, Myriam Oger, Jean-Jacques Michels, Benoît Plancoulaine, Paulette Herlin
France

Combined with image processing techniques, Virtual Microscopy can help pathologists in their daily practice to find objective criteria for differential diagnosis. Many works try to develop computer-aided diagnosis systems; one way relies on unsupervised clustering and classification algorithms such as diffusion maps but this technique is a CPU intensive process that has no straightforward extension for out-of-sample cases. This study focuses on this problem with the Nyström formula and illustrates the methodology for histological types of breast cancer.

A visual model approach to extract regions of interest in microscopical images of basal cell carcinoma

Ricardo Gutiérrez, Eduardo Romero
Colombia

Introduction
Recognition of regions of interest (RoI) in microscopical images may be a potential source of knowledge in many diagnostic tasks, it also may improve tasks such as medical education, medical training and diagnosis assistance. In addition, revealing such regions can highly reduce the computational and transmission charge of informative regions of a sample. Automatic recognition of such regions is really a harsh task because of the inherent randomness of the tissue's cutting and orientation. In spite of these difficulties, the pathologist's visual system efficiently recognizes regions of interest in several image domains by fusing image and task dependent information into a unique framework. This paper proposes a novel automatic approach to recognize RoIs by emulating the processing of the human visual system (HSV), not only modeling the preattentional process but also integrating it with high level processes.
Method
The approach proposed herein models the function of the HVS in four steps: firstly, it assigns a local relevance by integrating information from basic features as orientation, color and intensity at multiple scales, as previously described by Itti (V1 cortex function). Secondly, the model segments the image by taking into account the proximity and similarity between pixels and mixing up the conspicuity maps into the resulting regions (V2 cortex function), but also adding a map of the intrinsic structural disorder which models the specific task knowledge that regulates the attention over each structure (V4 cortex function). Then, this intermediate map is used to feed a module that efficiently looks for basal cell carcinoma by comparing the pattern composition with a data base of carcinoma regions, starting from the most attentional ones (it inferotemporal gyrus function). Once the algorithm sets up the first carcinoma region within the image, the other carcinoma regions are defined as the most similar regions using an Euclidean metrics of the different basic features: color, entropy and image intensity.
The model was tested with a total of 95 histological microscopical fields of view of different types of basal cell carcinoma, sampled from 25 randomly chosen patients, were selected for this evaluation. The set of evaluation was composed of microscopical fields taken at different objective magnifications. Each biopsy was formalin-fixed and stained with Hematoxylin-Eosin dyes. An expert pathologist, with at least five years of experience, selected the digitized fields of view and manually segmented relevant regions.
Results
The method herein presented was compared with a previous one proposed by us that outperformed the state of the art. The addition of the inferotemporal gyrus function improves the RoI selection. The sensitivity and specificity obtained by our method when applied the whole dataset was of 81% and 64% respectively.
Discussion: This paper presented a novel methodology to find RoI based on the human visual system. This differs from our previous approach by the inclusion of a stage of region recognition and evaluation of inter-region similarity. These characteristics let us improve the RoI extraction since the selection criteria are modified by a knowledge database.

The Metrizer: an innovative device for achieving virtual hepatic biopsies

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Italy

Background
The last few years have brought rapid growth in the number of Virtual Microscopes and the promotion of digital histology. The advanced technology herein proposed aims at being a sophisticated imaging archive. Digitally scanning slides however, does not give additional information to the vision of tissue structures, even with high resolution or improved colour and image precision.
The aim of this study is to present and validate a machine invented to facilitate the work of the observer in a medical practice, not only in terms of easy retrieval of images but also as an instrument for the automatic analysis of digital histology.
Findings
The "Metrizer" automatically supplies a description of that which it sees and then formulates what can be defined as a computerised diagnosis, generated by an expert system shaped on a series of data that has been preloaded once diagnosis is known. The automatic diagnosis permits to completely abolish operator subjectivity, from data capture to the print-out of final report. 435 biopsies taken from patients affected by HCV were tested to validate the machine.
All slides were analyzed automatically and diagnoses were compared to a previously validated system.
Conclusions
The Metrizer changes the method of examining liver biopsies since it resolves fundamental problems to measure microscopic structures. The automation of the Metrizer standardizes measurement and eliminates operator fatigue, it reads elements which are completely invisible to the human eye and has an unlimited calculation potential.

Computer aided diagnoses of sentinel node micrometastases

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Background
Careful analysis of numerous sentinel lymph node tissue sections is required to optimize the treatment regimen for individual breast cancer patients. Current research efforts of our group are directed towards automated prescreening of digitally scanned sections, both using H&E and applying immunohistochemistry (IHC). Aim of this study is gaining insight in the possibilities and limitations of this technique, applied to IHC stained slides.
Methods
Setinel lymph node sections (n=101) were stained by IHC to highlight possible micrometastases (MAb CAM5.2 and AE1/AE3) and digitally scanned. Subimages of micrometastases were manually extracted from scanned slides. Image segmentation was performed using colour deconvolution, and subsequently features were extracted describing geometry and densitometry of the IHC signal. A statistical classifier was constructed capable of automated identification of these events. Classifier characteristics were studied. Also, different methods for presentation of detected events were tested (gallery display, annotated virtual slide).
Results
AE1/AE3 showed better specificity compared to CAM5.2, which showed a high level of false positives. The area under the curve (AUC) for automated detection of AE1/AE3 stained cells was 0.89. The positive predictive value did not exceed 8% for any specimen. Nevertheless, prescreening was found to be valuable because only a limited number of events needed to be inspected visually, resulting in a significant time gain. Most probably, the most optimal presentation of detected events is a gallery with direct link to the virtual slide.
Conclusion
Computer aided diagnosis may be a valuable aid to diagnostic pathology. The present study shows the possibility of prescreening sentinel lymph nodes for detection of breast cancer metastases. Application of this technique enables assessment of an increased number of sections, increasing sensitivity of this method.

Computer-assisted Furman grading system applied for the clear cell renal cell carcinoma (CCRCR) analysis: a pilot study

Michał Kruk, Wojciech Kozłowski, Robert Koktysz, Stanisław Osowski, Tomasz Markiewicz, Janina Słodkowska
Poland

Introduction: Renal cell carcinoma (RCC) is an aggressive malignancy associated with a high rate of metastasis. Clear cell RCC (CCRCC) is the most common histologic subtype of kidney cancer. The Fuhrman grading system (FGS) based on nuclear characteristics is most widely used by pathologists and represents one of the most significant prognostic variables in patients with all stages RCC. Automated quantitative image system can be helpful in objective and accurate tumour grading to predict cancer-specific mortality.
Aims: The study was performed to design a software for the automated nuclear grading related to the conventional 4-tiered FGS for CCRCC analysis.
Methods and material: The designed FGS software is based on the combined methods of mathematical morphology, Hough transform and neural network for the microscopic digital images of CCRCC stained routinely with H&E. The procedure includes: segmentation of the individual cells ccRCC in the microscopic images from the image using the morphological and color-based operations, the preprocessing stages leading to the extraction of nuclei of carcinoma using Hough transform, features of nuclei extraction, selection of the most important features and the classification stage applying the neural classifiers.
The key points of the algorithm are:
• Hough transform applied to detect only round-shaped objects such as nuclei
• Fuhrman grade estimation using Support Vector Machine (SVM). SVM was used as a classifier to estimate Fuhrman grade on the basis of nuclei features.
The quantitative analysis was carried out on the collection of 100 CCRCC from the archive of the Pathomorphology Dept., the Military Institute of Medicine (Warsaw, Poland). Five representative digital images of each RCC were examined. The results were compared with the manual FGS measurement done by the same observer in the light microscope. Final statistical evaluation was applied.
Results: The preliminary results suggest an average discrepancy rate between the automated and manual reading scores as below 10%; the accuracy acceptable in the medical trials.
Discussion: The most important solved problems of our study include: the segmentation of the image to extract the individual cells, preprocessing of the cell images to generate the numerical diagnostic features describing cells nuclei, nuclei detection and Fuhrman grade estimation. The designed automated system has potentials of the standardized quantitative procedure and being improved classifier by adding other analytical features e.g. describing nuclei or modifying segmentation process to enhance classification.

A3: TELEDIAGNOSIS

Construction of web-based remote diagnosis system using virtual slide for routine pathology slides of the rural hospital in Japan

Ichiro Mori, Takashi Ozaki, Yasuteru Muragaki, Takatoshi Ibata, Hiroshi Ueda, Toshihito Shinagawa, Yoshiyuki Osamura
Japan

Background
The virtual slides (VS) of routine H&E stained pathology slides from a rural hospital were diagnosed through internet from Tokyo, about 500 Km from the hospital.
Findings
We limited the target to the biopsy specimens, and scanned using 40x objective lens. Pathology technicians store VS to their server together with clinical application forms. Pathologists open shared folder through internet, make primary diagnosis, and store the report to the folder using remote VPN software. As a result, we could diagnose most of the routine HE slides. Because we used public internet network, pathologists could manage their time and location for diagnosis easily.
Conclusions
As a conclusion, web-based remote diagnosis system is useful especially to the Japanese rural area where we do not have enough pathologists.

Telepathology Consultation in China using Whole Slide Image and an Internet based Platform

Chen Zhou, Amir Rahemtulla, Tong Hua Liu, Rong Sheng Huang, Huaqiang Shi
Canada

Telepathology is a powerful tool for remote consultation, especially in developing countries. We reported the practical use of a telepathology consultation service in China using WSI on an internet-based platform. Representative tissue slides were digitalized into WSI by a Motic Virtual Microscopy Scanning System (Motic, China) and sent to the website, www.moticteleppathology.com for consultation. A list of 84 expert pathologists was available to choose from for consultation. Over the last 3 years, a total of 1022 pathology cases from 29 hospitals were sent to the platform. The average WSI per case was 1.8 and about 90% of cases had either one or two WSI. The average turn around time was 38 hours; 79% of cases were reported within 48 hours. The most commonly submitted cases were gynecologic, gastrointestinal and liver/pancreatic, and pulmonary. 810 (79.3%) of the cases were neoplastic. 302 (29.5%) out of 1022 cases were not provided with a preliminary diagnosis by the referring pathologist; and among 720 cases with a preliminary diagnosis, 122 (16.9%) cases had an expert opinion which disagreed with preliminary diagnosis. The results indicated that a telepathology service using WSI and an internet-based platform is easy to use, safe, has a fast turnaround time and can benefit referring pathologists with the correct diagnosis in 41.5% of cases they submitted.

B2: PATHOLOGISTS OVERSEAS

Special session from the Patologi Oltre Frontiera (Pathologists Overseas) association.

Telepathology in low-resource settings: advantages and limitations

Agostino Faravelli
Italy

In rich countries telepathology can be considered an optional feature, meant to improve the quality of medical treatment; in most developing countries, on the contrary, it is an opportunity in the service of survival.
Telepathology in one of the poor countries, when correctly managed, can contribute in a conclusive way to supply to human beings the sort of information that we take for granted: the diagnosis of an illness, the basic piece of information which makes it possible for any medical or surgical treatment to be set up.
From an even superficial inquiry into the situation of the 54 countries in sub-Saharian Africa, we realize that almost all of them can avail themselves of a laughable number of doctors whose specialization is surgical pathology and those few have no instruments and very often they have not even received the corresponding training. For the people of those countries this means the impossibility of receiving adequate treatment in the case, for instance of malignant neoplasm.
Telepathology then allows the production of remote diagnoses and, on another plane, the training in loco of medical personnel. The situation of surgical pathology in four African countries in which POF are active is the same as that of all sub-Saharian countries except South Africa.
The few pathologists to be found there are almost always located in the capital.
In which way telepathologists and tele-education could lend a hand in, if not solving, at least stanching this dramatic situation? The experience of POF in Zambia and in Madagascar can set a pattern of how the situation could be handled, although the problems to be faced and solved are still many. In both places operations have been performed along the following steps:
Choice of a hospital, appropriate to the project: it should have a broad-band connection to access the internet, particularly in upload
2) availability of at least two either lab-technicians or units of staff with training in nursing
3) training in loco of the latter young people in reading cervical smears. Once trained, these young people will be able to prepare and colour a cervical smear, as well as conclude by themselves negative cases and select positive or dubious cases. The latter will get photographed and the images get then uploaded on a programme on the net created on purpose by POF; on this the Italian voluntary pathologist can see the images and insert his diagnosis
4) the same young people get trained on the spot as histology techynicians to fix, itemize, include, cut and colour the histologic material. In the lab equipment a system is included that allows a pathologist located elsewhere, by means of an internet connection, to guide on line the technician's sampling
5) when the histologic preparations are ready, they get scanned, turned into virtual histologic preparations and moved to a server where the voluntary pathologist can observe them and complete a diagnosis
6) the macroscopic and microscopic descriptions and the diagnosis get inserted into the internet programme which contains all the patient's data
7) the updating of the personnel's training takes place by means of lessons by the same teachers who trained them originally
8) according to availability of one or more doctors, POF will be available to train as pathologists, in agreement with the department of surgical pathology of the local university and with the local government.
After this has all been done, some problems remain to be faced:
1) high cost of the internet connection
2) an attempt to give their due to local pathologists to whom the diagnostic activity is entrusted
3) the role of the POF in the diagnostic activity: quality control
4) the organization of bringing the local medical personnel's training up to date (the function of telepathology and tele-education)
5) legal medical aspects of remote diagnosis

Telepathology as a tool, from diagnosis to quality control

Stefano Guzzetti
Italy

Traditionally, those who make diagnoses with a microscope are brought to share their opinions among their colleagues, especially in difficult cases.
A microscopic diagnosis is often a collective diagnosis, the result of the experience of several pathologists, each of whom contributes to the drawing up of the report according to his/her own experience. In addition each pathologist will then be able to enrich his/her own experience after each exchange of views with colleagues.
This need to share is not only suggested by the aim of avoiding legal issues, but also by the awareness that every patient deserves a detailed and correct diagnosis and that such a diagnosis cannot be based simply on the experience of a single observer.
Telepathology has allowed us to dramatically increase the possibility of exchanging diagnostic views, crossing out distances and allowing contacts among pathologists all over the world. Consequently telepathology has emerged as an essential diagnostic tool in those parts of the world where no pathologists are to be found or where their number is very small or else where they are concentrated in in health facilities which are few and far between.
The advantages of offering the benefits of histologic and cytologic diagnoses even in low-resource settings are reflected primarily on treatment plans (optimization of available therapies, often few and expensive), but also on preventive medicine (possibilities of setting up screening programmes in areas where the shortage of specialists doubles the high incidence of neoplasms that could be eligible for such programmes).
The reliability of diagnoses made on the so-called "virtual slide" is well documented: it is now time to develop a project with the aim of using telepathology to optimize the scanty human resources available in developing countries, joining the local pathologists by means of a network of telepathology stations. Thus, even small hospitals scattered over vast areas and far-away from each other could exploit the benefits of reliable pathological diagnoses.
In such projects, the role of pathologists from developed countries could be limited to supervision and to a further quality control over diagnoses completed by the network of pathologists in developing countries. Finally, the development of e-learning platforms could contribute to a continuous and regular professional updating of those same pathologists.
From an innovative experiment in a short time telepathology has become a powerful tool for spreading pathology in places where it seemed impossible to suggest it and will certainly contribute to an overall growth of the health condition of large parts of the world where the tools for diagnosis and treatment are still affected by the scarcity of resources and operators available.

Challenges in establishing a digital pathology facility in Pakistan

Yukako Yagi, Imtiaz Qureshi, Anurag Sharma, Asif Zafar Malik, David Wilbur
USA

Introduction:
Pakistan is one of the most densely populated countries in the world. However, the number of doctors in the country is not commensurate with the number of patients. The doctor to population ratio is 1: 1555 and there is one specialist per fifteen thousand people. When it comes to pathologists, the situation is even worse and there is serious shortage of pathologists even at tertiary care hospitals. Therefore, this project has been started in 2007 to implement a modern digital pathology facility at Holy Family Hospital (HFH), Rawalpindi Medical College, Rawalpindi, Pakistan working with a group at Massachusetts General Hospital (MGH), Boston, US. However, because of political tensions around the world, we had to modify the project plan to fit with the current challenging situation. The project goal was changed such that MGH would support HFH, which in turn would support other facilities in Pakistan using the digital pathology to improve overall pathology practice in Pakistan. To reach the goal, we focused on 1. Providing technical training in digital pathology and pathology practice; 2. Establishing a network between pathologists in HFH and MGH.
Methods:
A pathologist from HFH spent two weeks at MGH for training in digital pathology, pathology diagnosis, and information technology (IT) with hands-on experience in a variety of whole slide imaging (WSI) systems for scanning and viewing, experience with WSI based analysis, installation and trouble shooting of the WSI scanner which will be installed at HFH, and simulation of WSI-based and static image-based teleconferencing. As part of the training, attendance at sign out sessions and conferences at MGH was included. Prior to the pathologists visit, MGH prepared all equipment for the HFH pathologist; including a multimedia computer, tablet, and storage and ensured installation; and provided testing of all required software.

After the pathologist returned to Pakistan with all necessary equipment,, a weekly teleconference between MGH and HFH has been scheduled using GoToMeeting, Citrix Online, CA, USA.

Results:

The training sessions for the pathologist from HFH were very successful. Starting in December of 2011, a weekly conference for 1 hour between MGH and HFH has taken place. The pathologist at HFH presents 7-10 cases (total 34 cases for one month) using PowerPoint. Types of cases cover a wide variety because a pathologist in Pakistan has to diagnose all types of cases. All sessions have been recorded.

Discussion:

It was most useful to have the HFH pathologist at MGH for two weeks of training. To learn about differences of culture, pathology practice, IT environment, and having communication directly helped us to continue the teleconference weekly and made it easier to provide support from US. If the pathologist at HFH has a technical problem, support from MGH is improved because of familiarity with the PC, its configuration and with all installed software. This avoids other experience with other developing countries who could not download and install the software in their PC because of technical and network problem. We have yet to send a scanner to Pakistan; however, there is now a continuous working relationship between MGH and HFH, which has been fruitful for both sides.

Acknowledgement:

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C2: VIRTUAL MICROSCOPY

Virtual Microscopy with Google Earth: a step in the way for compatibility

Luis Alfaro, Maria Jose Roca, Pablo Catala
Spain

New developments in the field of virtual microscopy appear continuously. However a wide variety of image formats, software viewers and servers challenge compatibility in the managements of virtual slides. Google Earth is a well-known software oriented as a geographic information system working in a way similar to virtual microscopy, zooming and panning images, and moving along huge files.
With the aim of testing the value of Google Earth as a software for the handling of virtual slides we selected 20 pathology cases. They were scanned with a·3D-Histech Panoramic Midi, and an Aperio XT. Original virtual slides were exported into .jpg flat files with Aperio ScanScope software. We generated kml files, the file format to display information in Google Earth, and the pyramidal tiles structure compatible. The software employed was GDAL, an open source library for geospatial data images, with the utility gdal2tiles, and its graphical interface variant (MapTiler).
Cases were uploaded into servers, and were accessible directly through its web address and opened directly with Google Earth. All functions to allow diagnostic, consultation, educational... purposes were available. Image quality obtained was equivalent to original virtual slides.
Google Earth can be a good choice to avoid compatibility limitations in virtual microcopy. Viewing slides with Google Earth requires not technical skills. Exporting virtual slides and generating the tiles and files to serve them for Google Earth requires some knowledge in information technologies, however it is possible for pathologists to do without technical assistance.

JP2 WSI Converter: a universal JPEG2000 virtual slide conversion tool

Vilppu Tuominen, Jorma Isola
Finland

INTRODUCTION: Virtual slides (or whole-slide images) are becoming widely used in diagnostic pathology. At the same time, the capacity of automated slide scanners has also increased significantly. However, due to vendor competition, there is currently a plethora of closed, proprietary virtual slide formats, which are non-compatible with each other. We have previously shown that JPEG2000 is a viable option as a universal format for virtual slides, offering an open and standards-based alternative. However, JPEG2000 is sometimes criticized for its computational complexity and slow data compression. In this study, we describe a novel software application, entitled JP2 WSI Converter, which is designed to convert most existing scanner vendor formats into JPEG2000 in a 64-bit, multi-CPU environment, thereby enabling rapid conversion speed and integration with routine slide processing workflow.
MATERIALS & METHODS: The software development was based on vendor-specific libraries covering their respective formats, such as MRXS by 3DHISTECH, NDPI by Hamamatsu Photonics, and Surveyor SWS by Objective Imaging. To further improve the virtual slide format coverage, the academia-based OpenSlide library was also embedded into the software. In addition, the software uses several standard imaging libraries, such as libjpeg and the BigTIFF version of libtiff, and the JPEG2000 conversion is made using the ERDAS ECW JPEG2000 SDK. The conversion performance was measured using virtual slides of various size and content on several different workstations, while monitoring the performance metrics of CPU, RAM, and hard drive.
RESULTS: The developed JP2 WSI Converter produces JPEG2000 files with optimal code-stream parameterization for virtual microscopy, which we have described in our earlier study. The software is targeted for Microsoft Windows® operating systems, featuring a command line interface for batch usage and a graphical user interface with the option to process either a single file or a whole directory of files in sequence. Our tests show that the conversion scales well with the number of CPU cores, is not hindered by the hard drive performanceespecially when using fast solid-state drivesand does not require significant amounts of RAM. The JP2 WSI Converter is publicly released for free usage on our research group website http://jvsmicroscope.uta.fi/.
DISCUSSION AND CONCLUSIONS: We have described a universal conversion tool readily capable of importing the virtual slide data from most existing virtual slide scanners and converting it into JPEG2000. Our test data shows that the computational complexity of JPEG2000 is not an issue in multi-CPU environments, thereby allowing the software to be directly linked with a routine slide processing workflow. We anticipate that JPEG2000 will continue to gain momentum in virtual microscopy and ultimately become the standard format for virtual slides.

Hosting and Managing Large Sets of Virtual Microscopy Slides on Content Management Platforms on the Web

Hans-Peter Sinn
Germany

Introduction The presentation of virtual microscopy on the intra- and internet requires the availability of tools that act as a bridge between the repository of digital slides and a content management system (CMS) for the web presentation. Usually, this involves specific programming of the CMS in order to display the virtual slides in a consistent and specific manner. This is true for E-Learning systems, but also for digital slide repositories on the web. We have created several internet platforms for various purposes present some of the technologies that we for this purpose.
Methods: Educational slides were digitized using and Aperio CS scanner and stored in Aperios svs Fileformat. An load balancer was set up using the Apache reverse proxy technique in order to serve the slides to 70 students in a classroom simultaneously. The slides are managed by a Moodle 1.9 E-Learning platform, making use of the database module and customized javascripts for the presentation of the slides. For non-educational purposes, the Textpattern CMS was successfully employed for the presentation of digital slides on the Internet. Three such sites were set up, the largest hosting some 5600 digital slides.
Results: The use of Moodle and Textpattern as Content Management platforms turned to be very successful for the hosting of hundreds and thousands of digital slides on the internet. Slide series were easily categorized into sections and sub-categories, and linked to additional case material, such as clinical data, diagnosis, histological descriptions and PDF handouts. Thumbnail images are created on the fly using Aperios ImageServer software.
Discussion: E-Learning systems and content management systems can be adapted for the use of the presentation of digital slides for presentation on the intra- and internet without resorting to commercial solution. In this way, the imaging content can be easily linked to other case material and presented in an individualized fashion with relatively little effort.

Evaluating visual explorations of virtual histopathological slides through a new topographic measure for the complexity of gaze paths

Patrizia Morbini, Marco Piastra
Italy

Introduction
The physicians visual experience is at the root of the diagnostic process in medical disciplines such as pathology and radiology.
Tracking the path that the pathologists gaze covers when analyzing histopathological sections is one of the methods proposed to understand the diagnostic process in pathology and to compare the performances of individuals with different degree of diagnostic experience.
Data collected with gaze-tracking instruments have been analyzed in terms of number and duration of fixations, number and length of saccades between fixation points, and position of fixations with respect to regions of interest on the microscopic section. Significant differences in these parameters have been observed depending on pathologists experience. No measurement method has been implemented, however, to analyze and quantify the complexity of the visual exploration path of a specific histopathological section. Measuring such a degree of complexity is believed to have a clear perceptive relevance.
Methods
We designed a measurement method taking into account both geometric and topographic aspects of the exploration path recorded through eye-tracking. This method, derived from a mathematical measure proposed in the field of software self-organizing networks, is based on Voronoi tessellations. Briefly, for each fixation, a Voronoi cell is defined as the set of pixels which are closer to this fixation than to any other. A Voronoi tessellation is the union of all Voronoi cells.
We tracked the changes in the Voronoi tessellation produced by the sequence of fixations as the visual exploration proceeds. In a simple and systematic exploration, the cell corresponding to the current fixation will be adjacent to the previous one. By definition, an epicycle appears in the sequence when the current fixation cell is topographically separated from the previous one. The proposed measure, defined as sequential topographic product (STP), is the fraction of adjacent saccades, as defined above, with respect to the total of saccades. Lower STP values indicate a more complex visual integration task. Visual memory decay was taken into account by considering only the fixations within a predefined backward temporal interval.

Seven variably experienced subjects underwent eye-tracking with a non-contact, computerized system based on an infrared video camera, during a 60 analysis of 10 different virtual histological slides at 10x magnification. Slides represented cutaneous melanocytic lesion of different severity. STP was assessed for each eye-tracking registration.
Results
Standard deviation analysis showed a limited variability of STP for each subject across the slide set. STP values tended to be lower in more experienced subjects. Optimal backward temporal interval could be also derived from STP variance analysis.
Discussion
STP can be used to measure the complexity of the visual exploration path during the analysis of histopathological slides. The limited variability of STP for each subject across the slide set suggests that it represents an individual characteristic. Our preliminary results show a statistical bias supporting a higher path complexity in more experienced subjects. Further studies can be designed to asses STP variability in the same subject during the learning process.

Computer aided navigation - Virtual Microscopy as a tool for block centric slide reading

Norman Zerbe, Karsten Schlüns, Peter Hufnagl
Germany

Introduction:
In clinical pathology several use cases exist where viewing of identical histological structures in different slices of the same block is conducive. This requires an alignment of slides, which is currently done manually by the slide reading pathologist as required and just in time. Furthermore, alignment information is not stored permanently. Neither in conventional nor in virtual microscopy an alternation of three or more slides by retaining a relative position is feasible. We address this issue and present a framework to read slides in a more comfortable and intuitive way using computer assisted navigation integrated into virtual microscopes.
Methods:
Whole slide images contain multiple distortions from different sources in the laboratory and digitalization process. In the proposed WSI interconnection model distortions are described by several layers to provide a normalized representation of tissue. Each layer is associated with a specific distortion and serves as a new level of abstraction. The interconnection model enables a coarse alignment of tissue sections. Further alignment of sections is done by multi-resolution feature point extraction and matching. Results are validated using RANSAC filtering and subsequent manual inspection and refinement as required. Corresponding image locations are stored in a database. Transformations from source locations to destination locations are calculated using the corresponding pairs. Inside the convex hull of all fiducial points a local affine transformation is applied whereas outside a rigid transformation used.
Results:
The implementation of the interconnection model stack uses REST web services to deliver normalized image data as well as metadata. In addition, a localization service provides a query interface to calculate corresponding image locations for an input location. The services are used in our whole slide image based tumor board meeting system.
Discussion:
Computer assisted navigation is a valuable tool to save time in routine pathology. Alignment of histological slides can be preprocessed and it is no longer assigned to pathologists. The proposed framework allows pathologists to examine tissue sections of the same block independent of laboratory and scanner related distortions. Hence, slide reading shifts from a slide centric towards a block centric perspective. Due to the heterogeneity of laboratories, slide scanners, and their integration further standardization with respect to the WSI interconnection model may be useful.

A4: INTERNATIONAL SCANNER CONTEST 2012

Evaluating Image Quality - First impressions of 2nd International Scanner Contest 2012 in Berlin

Thomas Schrader, Norman Zerbe, Peter Hufnagl
Germany

Based on experiences of the First European Scanner Contest the 2nd (now) International Scanner Contest took place at the Week of Pathology in May 2012 in Berlin. Scanner of various vendors were tested in different domains to compare and measure image quality.
The problem of image quality covers various requirements of pathologist: they want to have a very short scanning time as well as high quality of WSI. But the quality aspects of WSI are not well defined and depend on use case, experiences and expectations of pathologists.
The Scanner Contest has at least two main aims:
1. compare the scan results of various scanners,
2. discuss the aspects of image quality to establish a common sense about that.
In five different domains the scanner were evaluated autmaticall or manually :
1. High Throughput - 35 slides should be scanned automatically
2. Quality Scan - 10 slides should be scanned manually to get the best possible image quality.
3. Fluorescence Scan - slides with Quantum Dots and Flourescence Dots should be scanned manually.
4. Image analysis - Algorithms to quantify Ki67-stained breast cancer TMA dots were compared to a manual evaluation
5. Technical - Calibration slides for geometry and color were digitalized and analyzed to calculate differences and compare them.
During the contest different technical parameter were measured and compared such as scanning time and power consumption.
The test domains represent requirements in routine pathology, education, and research concerning advantages and weakness of the scanner for special use cases. Pathologists evaluated random selected areas of WSI and rank them and express their opinion about image quality.

A5: TRACEABILITY

Invited talk: Interoperability in traceability and digital slides systems *(M.Garcia Rojo, Spain)*

Data and image management in pathology can benefit from traceability systems. Implementation of tracking and traceability systems need close interaction between pathology information system (LIS), touch screens (light clients), cassette and slide label printers, slide stainers, imaging devices, and hospital information system, amongst others. Since no interoperability standards has been designed between LIS and traceability standards, implementation of this systems maybe cumbersome. Traceability systems allow for a unique identification of objects (containers, cassettes, and slides), using one or two-dimension barcodes. Identification of gross images and digital slides can greatly benefit from this, since manual data input is not needed anymore to identify digital images. Other benefits are improving workflow, timing management, avoiding identification errors, more reliable technical reproducibility, and object tracking.

We describe main benefits from current available traceability systems, focusing in our experience with Dako TruePositive ID (TPID), and describing other systems like Roche Ventana Vantage, Shandon SlideMate and ChessMate, and Leica Cognitive Cxi Printer and Universal Labels. All this solutions must adapt to the reality of each department. We review different scenarios, comparing institutions were electronic request of pathology studies is already working and those were pathology study request are made in a paper form; comparing those laboratories that work in a batch mode with laboratories that process specimens continuously.

Since pathology cases are frequently sent to other pathology departments for second opinion or ancillary techniques, a global unique identification for specimens, including identification of the institution, and a consensus in specimen labeling is requested. These topics are currently being studied by HL7 anatomic pathology special interest group and DICOM WG26.

A6: VIRTUAL MICROSCOPY 2

Larynx Virtual Slide Validation Study

Bart Sturm, Stijn Fleskens, Freek Bot, Marie-Louise van Velthuysen, Piet Slootweg, Jeroen van der Laak
Netherlands

Introduction
Whole Slide Imaging is a technology in which histopathological slides are optically scanned to produce digital images. So far, virtual microscopy (VM) has mainly been used for educational and research purposes. In surgical pathology, VM was found to be useful for frozen section diagnostics in remotely situated hospitals without a practicing pathologist on location. We expect a major future role for VM in consultation of specialized pathologists for difficult diagnostic cases. Aim of the present study is validation of VM for tissue samples of pathologic lesions in which subtle differences have major clinical implications. Larynx biopsies (n=106) were used containing pre-neoplastic lesions, which were assessed on glass slides in a previously published study, independently by 3 expert pathologists.
Methods
Specimens were obtained from the department of pathology, Radboud University Nijmegen Medical Center (n=60) and Maastricht University Medical Center (n=46). Glass slides were scanned using an Olympus dotSlide system with 40x objective. The virtual slides were displayed at a calibrated professional HP LED monitor by means of a HP ProBook notebook computer and Olympus OlyVIA virtual slide viewer. Calibration of the monitor was performed with a Datacolor Spyder 3 Elite colorimeter. The virtual slides were reviewed according to the 2005 WHO classification system by three pathologists with head and neck pathology as a field of interest who took part in the former study. These diagnoses were converted into a three grade system to approximate clinical management. One pathologist performed a second glass slide read. A consensus diagnosis based on glass slides was available from our previous study. VM diagnosis concordance rates were calculated. Kappa statistics were calculated to assess the degree of inter- and intra-observer agreement.
Results
The overall interobserver agreement is comparably low for both VM and glass slide diagnosis (unweighted kappa 0.38 and 0.39, respectively; weighted kappa comparable). The intra-observer (glass vs VM) agreement ranges from 0.41 0.48 and 0.52 0.57 for unweighted and weighted -values respectively. VM versus glass slide intraobserver agreement equals the glass slide intraobserver agreement. Concerning the consensus diagnosis, virtual slides show overall slightly less (although not statistically significant) concordance compared to glass slides.
Discussion
This study is different in respect to former VM studies in that larynx pre-malignant pathology diagnostics is in general difficult expressed by low kappa values in this and other studies. Diagnosing larynx pathology on virtual slides is comparably feasible compared to traditional glass slide diagnosis. Slight differences between glass slides and VM against the consensus diagnosis may be attributed to a lack of experience with VM. Future studies will have to prove this.

A rich internet application for remote visualization and collaborative annotation of digital slide images in histology and cytology

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Belgium

Background
Digital slide scanning is advancing the field of pathology and biomedical research, resulting in very large amounts of imaging data. From the computer science point of view, it is challenging to efficiently share, annotate and analyze such data due to their distinct geographical localizations, their high dimensionality, and their numerous sources of variability (scanning equipments, file formats, acquisition protocols, application domains, ...).
Findings
We integrated recent web technologies and generic methods to allow remote visualization and collaborative annotation of digital slides through the Internet. Using this tool we were able to build atlases of thousands of annotations related to cell and tissue types in lung cancer studies from two distant research laboratories.

Promising cell and tissue recognition results were obtained by integrating a recent content-based image retrieval algorithm.
Conclusions
This work proposes a new web-based tool to ease collaborative projects in digital histology and cytology.

An open-source, MATLAB-based annotation tool for virtual slides.

Riku Turkki, Margarita Walliander, Ville Ojansivu, Nina Linder, Mikael Lundin, Johan Lundin
Finland

Background
Image annotation is an important step in the development of automated analysis methods for digitized microscopy samples. Annotated areas (i.e. regions of interest) are used both during the training process and for evaluation of performance of an automated tool. For example supervised learning algorithms require a large number of training samples to adequately learn a model for a particular task. Current virtual slide collections on the other hand contains vast amounts of data and new tools are needed to perform image annotation in a virtual microscopy environment. The challenge is to extract areas of interest including labels from digital whole-slide samples in an efficient and easy-to-use manner.
Findings
We propose a MATLAB program that can be set to access a slide collection and annotate points of interest from the virtual slides. In order to use the tool, the image server should be equipped with basic server software making it possible to access the data.
Conclusions
We provide a solution that combines fast iteration and transition between data annotation and method development. The usage of the tool is demonstrated in a virtual microscopy environment (the WebMicroscope platform), but it is designed to be modifiable to different platforms as well.

Colon biopsy diagnostics may reliably be performed using virtual microscopy

Bart Sturm, Chella van der Post, Jeroen van der Laak, Ruud Clarijs, Ewoud Schaafsma, Johan van Krieken, Marius Nap
Netherlands

Background
Virtual microscopy using whole slide imaging (WSI) is a feasible alternative to optical microscopy, offering major advantages for pathology practice. The present study aims to prove reliability of this promising technique for colon biopsy diagnostics.
Methods
Colon biopsies (n=295) were assessed (in 7 main diagnostic groups) using both glass slides and WSI by 4 pathologists and 2 residents. Two of the pathologists, having extensive prior experience in WSI, scored the WSI biopies in a primary diagnostic setting. For each case the criterion standard diagnosis was defined based on glass slide diagnoses. Accuracy was defined as the percentage of concordance with the criterion standard. Kappa statistics were calculated as a measure of observer agreement.
Results
The overall concordance rates were 89.5% for WSI and 91.4% for conventional microscopy. The intraobserver (WSI versus glass slide) agreement was good to excellent, with kappa values ranging from 0.72 to 0.87 (mean 0.78) and was higher than that for different observers evaluating glass slides (interobserver kappa = 0.71). No significant differences were observed comparing a primary and secondary diagnostic setting.
Conclusion
This study showed good diagnostic accuracy and reproducibility for WSI, indicating this technology may be used for primary colon biopsy diagnostics.

B3: IMAGE ANALYSIS

A Simple Mathematical Model Utilizing a Topological Invariant for Automatic Detection of Tumor Areas in Digital Tissue Images

Kazuaki Nakane, Yasunari Tsuchihashi
Japan

Background
The number of diagnostic pathologists is significantly small in Japan with respect to the large number of clinical cases that require pathological diagnosis. Because of this individual diagnostic pathologists are overloaded in their daily work. To improve the situation development of computer assisted diagnostic system for a pathologist will be one of the effective solutions.
Methods and Results
We hypothesize that a simple mathematical model using the Betti numbers can characterize tumor areas in a given pathological tissue image. We applied the idea for digital images of biopsied colonic mucosal tumors and assessed its validity.
Conclusion
Our simple mathematical model was found to be useful to differentiate tumor areas from their normal counterparts and it will be used for a computer assisted diagnostic system for pathologists.

Semi-automatic Evaluation of Intraepithelial Lymphocytic Infiltrate in Duodenal Biopsies on Digital Slides

László Fónyad, Marcell Szász, Lajos Berczi, Béla Molnár
Hungary

Background. During the past years digital slides have become more and more accepted as the quality and speed of scanning have both been improved. Besides using digital microscope as just a replacement of the optical microscopes, there is a constantly developing field of digital microscopy: automatic and semi-automatic evaluation of various parameters on a digital slide using sophisticated image analysis algorythms of dedicated softwares. In our study we have investigated the usefulness of digital slides in the diagnosis of celiac disease, where the accurate CD3+ intraepithelial lymphocyte count is crucial for staging.
Methods. Altogether 24 duodenal biopsy samples of celiac patients with 12 negative controls were collected from our archives. After CD3 IHC reaction the slides were scanned (Pannoramic Scan, 3DH Ltd.), representative areas of the epithelium were annotated manually and the intraepithelial lymphocytes were automatically counted using the immunohistochemistry image analysis module of the Pannoramic Viewer (3DH Ltd.) software. The results were compared with the manual H&E slide based lymphocytic count of the primary diagnosis.
Results. An average of 260 objects per slide was detected by the software. (~6700 epithelial cells and 2700 lymphocytes.) The software distinguished positive and negative samples correctly in 32 cases (89%) using the Marsh-Oberhuber classification. 4 false negative counts occured, no false positive count were observed.
Conclusions. After analysing the sources of errors responsible for discrepancy, objective evaluation of CD3 lymphocytic infiltrate of doudenal samples could be achieved using dedicated softwares giving an useful additional tool to the armamentarium of pathologists, similar to the method used for the automatic evaluation of immunohistochemical reactions in breast cancers.

Automated segmentation of blood cells in Giemsa stained thin smear images

Margarita Walliander, Riku Turkki, Nina Linder, Mikael Lundin, Juho Konsti, Ville Ojansivu , Taru Meri, Ville Holmberg, Johan Lundin
Finland

Background: Automated separation of blood cells from the background in thin blood film samples is of importance in the process of cell counting and as a first step in image analysis of red and white blood cells morphology.

Findings: We developed a method for blood cell segmentation that can separate the foreground (i.e. the blood cells) from the background. It can be used in combination with other algorithms for detection of intracellular parasites like malaria and quantitation of infected red blood cells.
Also the detection and counting of the total number of white blood cells in a whole thin blood film can be used for further differential counting.
Conclusions: We present an unsupervised tool for separating the foreground in Giemsa stained thin blood films and an automated cell counter for red and white blood cells. The segmentation of blood cells in thin blood films can be used as a pre-processing step to specify the regions of interest for a secondary algorithm, e.g. for image analysis of red and white blood cell morphology or detection of intracellular infectious agents.

Semiautomatic FISH quantification on digital slides

Gabor Kiszler, Tamas Micsik, Daniel Szabo, Laszlo Krecsak, Tibor Krenacs, Bela Molnar
Hungary

Background: Fluorescence in situ hybridization is a widely used diagnostic procedure in pathology. This method can reveal the genetic background of malignant lesions. The aim of our study was to develop and optimize an image segmentation algorithm specifically for FISH quantification in breast cancer tissue. Moreover, we aimed to validate the results of our algorithm and to compare them with a semi-automated assessment (i.e. scoring on a computer screen) and the results of the conventional (i.e. manual microscopic) IHC quantification.
Findings: Our system was tested on Her2/Cep17 labeled breast cancer samples, selected from the archived cases of the 1st Department of Pathology of Semmelweis University in Budapest, Hungary. The algorithm was integrated into the Pannoramic Viewer software (3DHISTECH Ltd.). The quantification of the virtual slides scored by the pathologist on the computer screen was run in parallel with the software measurements. The performance of the application was tested against the pathologist's scoring of digital slides on 39 TMA core covering all ICH HER2 score ranges, and the Cohen's kappa provided almost perfect agreement ($\varkappa$=0.911) among the two results.
Conclusion: We developed our own image segmentation algorithm consisting of two different detection methods: one specifically for the cell nuclei detection and one for spot signal detection. The input of the cell nuclei detection module was the DAPI channel. The detection was based on the intensity and the morphological characteristics of the cell nuclei, whereas the spot detection is performed based on the intensity peak of the spot signal. We integrated an object classification module to the application, which classifies the detected cell nuclei according to the suggested role of the applied FISH probe. Our preliminary results showed that the image segmentation procedure applied was suitable for FISH quantification. The automated diagnostic process was more standardized with the algorithm compared to the conventional diagnostic process. Finally, we have generated a software solution which could be a helpful tool for pathological diagnostics.

C3: DIGITAL STANDARDS

Tracking and Analysis of Business Processes in a Pathology Department

Thomas Schrader, Hagen Woecht, Stefan Krumnow, Roland Pauli
Germany

The development and application of Whole Slide Images (WSI) influence the pathology domain in general. Different areas benefit from this process. Now it is useful as well as necessary to describe and model all processes in a pathology department to integrate digitalisation and analysis of WSI. Furthermore the digitalisation of pathology related processes opens the possibility to share resources and knowledge. New terms circumscribe these aspects: digital and collaborative pathology.
Especially the activities of IHE (Integrating the Healthcare Enterprises) Working Group Anatomic Pathology promote the standardization process and enforce the exchange of data and knowledge based on communication standards. The Working Group together with the European Project COST Action IC0604 "EuroTelepath" described and modeled business processes in pathology using the standard BPMN (Business Process Modeling Notation). A logical consequence of these activities the models should be evaluated and analysed in reality of a pathology department: in a pathology department (Pathology Department of City Hospital Brandenburg) a case tracking system using barcode readers was implemented.
The general diagnostic of the COST Action Project modeled in standard modeling language Business Process Modeling Notation (BPMN 2.0) was adapted using the web based modeling tool by Signavio. This model was extended with special data entries to store process tracking data. The special tracking software "nomic" by InnoCon-Systems was used to track the cases during the flow through the department. During February to November 2011 the diagnostic process of all cases were measured. In the the general pathology process 6 points of measurements (PoM) were defined and barcode readers were installed at the working places of these process stages.
The data of observation period was analysed and allows a deep view inside the pathology processes. The reality of the diagnostic process was compared with the model processes of COST Action IC0604 to detect discrepancies, and to discover the benefit of application of business process models.
Business models are the basis for process tracking and process analysis. At least two aspects are important for the analysis of real process data collected during the routine workflow: impact analysis (how important is a process task for the complete process) and transformation analysis (consequences of changes). It helps to understand essential process steps, meanderings and the reasons for that. This is an very important information for improving the processes and plan resources in a pathology department.
The basic model from COST Action was marginal changed and extended to store real process data. A specialized software for business modeling (Signavio) and tracking (nomic) supports the process monitoring directly. These data can be used for further analysis and simulations of process changes.

A unifying diagnosis coding system for the structured pathology report in Italy

Arrigo Bondi, Paola Crucitti, Stefania Lega, Paola Pierotti
Italy

Introduction
The reports of Pathologists contain critical information for health, epidemiology and care quality monitoring, the systematic registration of which has relevance to clinical governance.
Traditionally, the pathology report is written in a descriptive form and it is often associate with a concise and essential coded information that include the body site sampled (topography), the main microscopic diagnostic conclusion (morphology) and sometime the procedure applied.
However, even these essential encodings are useful for epidemiological purposes, especially targeted at cancer registries: a common coding system is anyway mandatory.
The SNOMED and ICD-O systems are widespread international encoding methods available for pathologists but many local management systems adopted an inner table or subsets derived from partial autonomous translation of the original nomenclature. The result is an efficient system for local purposes only and a Babel of coded languages when one attempt to share data.
The ICD-O code is freely available for non-commercial use but it doesnt cover all the needing of a pathologists routine; the SNOMED code is subject to copyright by IHTSDO and it is available for use upon an agreement with a national Government delegate, but Italys Ministry of Health is currently using different international

coding systems and it is not planned a short term adoption of SNOMED. Furthermore SNOMED is going to be merged into the ICD 11 project by the WHO committed study group.
Methods
The National Scientific Society of Pathologists (SIAPEC-IAP) aware that the diversity of nomenclatures and diagnostic coding systems is a critical problem at national level, supported a plan to build an Italian Nomenclature for Anatomic Pathology (NAP), compatible with the international coding systems.
The Italian Association of Cancer Registries (AIRTum) and the WHO Italian Collaborating Centre for the Family of International Classifications have contributed to the NAP.
Results and discussion
This NAP terminology has been built extracting and unifying codes and terms from many databases currently used in Surgical and Anatomic Pathology Units in Italy, integrating them with the full version of ICD-O 3.
It is totally exhaustive for the oncology codes while in the other fields accomplish to the themes and codes that have been used at least once and it is included in one of the databases considered. This strategy may lead to an incomplete nomenclature but all the system is planned to merge into the IHTSDO project or into ICD 11 when available: they will ensure the completeness of the vocabulary.
The NAP include about 20,000 terms and it has been experimented for one year in all the Surgical Pathology Units of the Umbria Region, with no practical problems.

Impact of terminologies for tumor pathology structured reports

Gunter Haroske, Thomas Schrader
Germany

Introduction:
For information exchange and data mining structured reports in tumor pathology have to be based on controlled vocabulary as to get a model of meaning. So far there is no universal terminology for the wide variety of concepts in tumor pathology. SNOMED CT will probably become a global health terminology standard. National and international initiatives are necessary to reach a growing agreement on particular aspects and needs towards it. Interface terminologies are a tool for drawing existing separate terminology systems to a finally global standard.
Methods:
Controlled vocabularies in guidelines of German pathologists for a series of tumors, in the basic tumor documentation of cancer registries, and in the HL7 Germany have been mapped to PathLex, an interface terminology of IHE.
Results:
On average a pathology guideline describes 50 terms which have to be registered as to fulfill the minimum documentation requirements. PathLex provides between 30 to 40 terms per tumor entitiy, only 80% of them are identical with the German guideline vocabulary. The coincidence of PathLex with HL7 Germany vocabulary or the basic data set of cancer registries is still lower. In contrast to PathLex there is no separation between general and organspecific information in the German guideline vocabulary.
Discussion:
Although based on internationally agreed understanding, sharing the same concepts of tumor pathology, the terminology differences among the different sources are quite obvious. Those differences have to be overcome as to ascertain a reliable information exchange between different actors in the care of tumor patients. Terminology mapping is one solution, but not the optimal one. A closer collaboration with international terminology bodies as well as a sharpened realization of the impact of terminology in home made guidelines and beginning with ontology construction would contribute to a better standing of German pathology.

Integrating guidelines-based structured reporting into patient data management system.

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Croatia

Background: Driven by recent developments (socioeconomic situation, new technologies and drugs, molecular pathology, omics etc.) the possible impact of pathology in the healthcare system changes rapidly. Adaptation to a new role as clinical collaborator, diagnostic consultant, and physician leader (as pointed out by CAP) has become the imminent goal. In this different tools known already for a certain time, and discussed on an more academic basis are finding its way into everyday life. So is also the concept of structured reporting in Pathology. Most pathologists include into their reports, created usually as a descriptive free text, also elements from a checklist suggested by a given society or working group. Despite the summary of these data in the diagnosis and

closing remarks they remain mostly poorly organized and hard to retrieve/compare during search on later occasions. In a time where we need more and more detailed data on the disease of an individual patient followed trough time and also need to integrate them with other patient data in order to facilitate meta-analysis, creating structured reporting protocols seems to became an important goal. In contrast a structured report captures all of the elements across all sections of the report e.g. clinical, macro and microscopic, and uses narrative where ever necessary to expand or explain those elements. Different approaches have been tested in order to enable the most useful combination of the classical full text report and the sought for goal of structured reporting. Recently working groups dedicated to Anatomy Pathology within multiple standards organizations defined standard-based data structures for Anatomic Pathology reports and images as well as informatics transactions in order to integrate Anatomic Pathology information into the electronic healthcare enterprise.
Objective: to facilitate the exchange of structured pathology reports within the institutions of the healthcare system in Croatia and their integration with other patient data (e.g. images) as part of the Clinical Path.
Methods: The Croatian Society of Pathology is developing National guidelines for cancer reporting. All elements of the Clinical Path connected with tissue sample analysis performed by pathology departments (including clinical, morphological macro/micro and molecular data) are checked on a preformatted organ specific hierarchically organized electronic form included in the patient electronic examination record and saved into specialized Pathology information system (PIS) and pathology PACS (being the part of HIS) for later retrieval and analysis. For distribution purposes these forms are automatically converted into grammatically and syntactically correct free-text-like reports.
Results: Development of a PIS/PACS-based workflow and data management system including structured pathology reports. Eventually a nationwide network enabling exchange of structured reports incorporated into integrative patient database will be created.
Conclusion: The success and usefulness of medical IT systems in the next future will depend on their capability of integrating as many features as possible in order to enable an organized influx and trough this, applicability of the plethora of newly produced data.

A8 DIGITAL IHC 1

Digital Immunohistochemistry: New Horizons and Practical Solutions in Breast Cancer Pathology

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Introduction
Immunohistochemistry (IHC) has become a backbone of diagnostic and predictive pathology along with the more basic techniques of histology and histochemistry. While standardization of IHC staining protocols can be achieved by improving antibodies, detection systems, and automation, other aspects of the test remain more variable. The sources of potential variation span from the many steps of tissue processing to a visual evaluation of the slides by a pathologist. Virtual microscopy opens new opportunities to standardize the IHC test, especially, where visual evaluation can be supported by the digital image analysis (DA). In addition, new horizons of retrieving more useful information from the IHC test are opening by application of multivariate analyses of the data retrieved. We therefore evaluated potential advances of the digital IHC test in clinical and research settings.
Methods
Two aspects of the digital IHC test improvement were explored. First, implementation of digital IHC tissue controls replacing conventional evaluation performed by a pathologist. Tissue controls for routine breast cancer IHC testing, represented by a multiblocks, were scanned and analyzed on daily basis. The data were then compared to routine pathologists evaluation of the tissue controls. Second, the potential of factor and cluster analyses was explored to obtain new quality of information from the IHC data. Aperio XT scanner and image analysis tools (the Genie, Nuclear, and Membrane algorithms) were used.
Results
DA of daily and batch-to-batch variation in HER2 IHC tissue control results provided a robust monitoring system to alarm on unexpected changes and detect stepwise deterioration of the IHC staining and/or the control tissue. If serial sections of the tissue controls were used, quantification of several parameters (absolute and relative numbers of tumor cells with various staining intensity) with the same algorithm provided a set of indicators of stability of the test. DA of multiple IHC markers performed on breast cancer tissue provided a set of continuous variables for multivariate statistics. Analysis of the data enabled to extract biologically and clinically meaningful information opening a perspective of developing integrated biomarkers of the disease.
Discussion
While most immediate application of the DA in IHC lies in decision support when quantification of biomarker expression is desired, our experiments target other aspects that can enhance the IHC test. The first aspect is very practical and employs the potential of the DA to monitor the quality of the IHC test on daily basis, especially, when therapeutic targets are considered. It also provides efficiency exceeding and diminishing the need for routine semi-quantitative evaluation by a pathologist. Digitized IHC tissue controls can be easily accessed by all pathologists if needed. The second aspect employs mathematical processing of the DA data to explore intrinsic interdependencies of the IHC biomarker expression, the advantage largely impossible without DA. This opens new horizons for pathology research and development of complex tissue-based biomarkers.

ImmunoRatio-F: image analysis of ER, PR, and Ki-67 immunohistochemistry using cytokeratin immunofluorescense correction

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INTRODUCTION: Accurate assessment of estrogen receptor (ER), progesterone receptor (PR), and Ki-67 is essential in the diagnostics of breast cancer. Increasing number of pathologists and laboratories analyze ER, PR, and Ki-67 by using digital image analysis. We have previously published ImmunoRatio, an open source image analysis application, which has become widely used (web application used for over 25,000 analyses by 2/2012; Tuominen VJ et al. Breast Cancer Res 2010). ImmunoRatio, as well as many similar commercial software packages, calculates the percentage of positively (brown) stained over total nuclear area (brown+blue) by using the color deconvolution algorithm. Although ImmunoRatio provides accurate prognostic information in breast cancer (when analyzing Ki-67), the labeling index should be analyzed only from the malignant epithelial cells,

while excluding all stromal fibroblasts and lymphoid infiltrates. In this study, we describe a modification to ImmunoRatio, entitled ImmunoRatio-F, which is capable of solving the specificity problem by using a cytokeratin immunofluorescense correction.
MATERIALS & METHODS: A test set of breast cancer samples was double stained by mixing a mouse monoclonal cytokeratin 19 (CK19) antibody with the rabbit monoclonal Ki-67 antibody working solution. The detection steps consisted of anti-mouse Alexa 488 (=green fluorochrome) mixed with anti-rabbit peroxidase polymer, followed by diaminobenzidine (DAB) and hematoxylin counterstain. The stained slides look identical to conventional single-color immunohistochemistry samples under brightfield illumination, but appear as immunofluorescence samples under darkfield illumination. The test set was digitized with an automated microscope by sequentially capturing brightfield and green fluorescence images.
RESULTS: The modified ImmunoRatio-F software uses the fluorescence image (for CK19) to create an epithelial-cell mask for the analysis of the brightfield image, and is therefore able to restrict the labeling index quantification only to the malignant epithelial (carcinoma) cells. In our tests, ImmunoRatio-F improved the analysis accuracy compared to ImmunoRatio when using visual cell counts as golden standard. ImmunoRatio-F is open source and publicly released on our research group website http://jvsmicroscope.uta.fi/.
DISCUSSION AND CONCLUSIONS: Our study shows that a double stain with immunofluorescence and peroxidase detection is a feasible solution to improve the accuracy of ER, PR, and Ki-67 image analysis. The staining components do not interfere with each other, as is the case when using chromogenic double stains. This methodology can be applied broadly in diagnostic pathology, for example, in the analysis of prostate cancer (AMACR+Ki-67) and lymphoma (Ki-76+CD3/CD20) samples.

A multistep image analysis method to increase automated identification efficiency in immunohistochemical nuclear markers with a high background level

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Background: The “removal” of background from digital images (DIs) to identify only the objects of interest is difficult due to the overlapping color values of pixels of the nuclei and background. We outline a new automated procedure to quantify only immunohistochemically stained nuclear markers, despite the similar color of the background. This procedure was compared with the gold standard manual method and a previous method developed for low-background DIs.
Findings: The automated multistep DI analysis procedure integrates the selection of successively smaller ranges of brown pixels, several ranges of area and roundness parameters and the use of different masks. At the end of the process, an algorithm separates each DI for subsequent application of the appropriate macro for optimal quantification. For high-complexity DIs with background, the quantification of nuclear markers obtained with the new procedure is closer to the gold standard than that provided by the old macro. For high-complexity DIs without background, the two automated methods give the same results. The presence or absence of background does not appear to influence greatly the quantification of nuclear markers in low-complexity DIs.
Conclusion: The selective identification of brown color ranges and morphological parameters in DIs provides better background discrimination during the automated quantification of positive nuclear markers. Its implementation is straightforward, uses relatively widely available software and is applicable to all quantitative nuclear signals from a variety of tumor specimens.

Is Her2 amplification predictable by digital immunohistochemistry?

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Background: HER2 positive breast cancers can benefit from trastuzumab therapy based on a validated immunohistocemical reports. HER2-negative and strong positive cases are easy to interpret, but equivocal cases should be analyzed with FISH-technique to reveal HER2 amplification. Image analysis methods have been recently developed such as MembraneQuant by 3DHISTECH to support this process. We validated MembraneQuant software on HER2-immunostained (clone 4B5) tissue microarrays of 100 breast cancers covering all positivity groups and tested if semi-automated software analysis of HER2 immunostaining can discriminate between FISH-positive and negative equivocal cases. The renowned 4-tiered evaluation guidelines were used. The HER2 gene status of the 15 equivocalcases was also assessed with FISH. Detailed MembraneQuant analysis in the 9 FISH- and 6 FISH+ cases was used to predict HER2 amplification status.

Findings: We found excellent agreement with membranous (Quadratic weighted $\varkappa$=0.962, Range 0 to +++) staining between the results of the digital and semiquantitative evaluations. By detailed digital analysis of equivocal cases, there was a trend towards lower immunonegative cell number and higher 2+ cell number in the FISH- cases, while FISH+ cases had significantly higher 3+ cell number and H-Score.
Conclusion: We found MembraneQuant application to reliably analyze Her2 immunostainings according to latest guidelines and classify cases into various groups in good correlation with the semi-quantitative evaluation. Furthermore, MembraneQuant could help to differentiate between FISH negative and positive cases, and thus to predict HER2- amplification without FISH facilities. Furthermore, MembraneQuant offers itself as tool for archiving, documenting and standardizing immunohistochemical evaluations in line with recent quality initiatives.

B4 QUALITY IN DIGITAL PATHOLOGY

The Image- and Data Quality Ontology for scientific data management

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The Open European Nephrology Science Center (OpEN.SC) as a center for research relevant clinical data discussed the topic of data quality very early. Data quality is a multidimensional entity, represents different aspects such as process or service quality, and depends on use case and purpose. But images have partially the same dependencies in quality related to use case and quality of service.
A Data Quality Framework based on a Service Oriented Architecture was suggested including an ontology for image and data quality.
Technological view: the OpEN.SC platform based on a Service Oriented Architecture with various web services. The backend is an Apache Geronimo-Application Server, the Open Source database engine PostgreSQL is used to store the data from three different resources. The user interface is realized by OpenSource Liferay Portal. Protege was used to model the ontology.
Methodological view: To create the ontology the Delphi-Method was used. Quality criteria, measurements based on current literature and reflects the guideline for scientific data management of TMF in Germany (Technologie- und Methodenplattform für die vernetzte medizinische Forschung - Technology, Methods, and Infrastructure for Networked Medical Research). New measurements and describing properties were developed for data as well as for images and integrated into this ontology.
The ontology reflects the complete process of data management including the various roles and relations of persons to the data and images. The ontology covers 18 different quality categories with various concrete aspects and measurable characteristic numbers. They are related to use case, application area and responsibilities and tasks of persons who are involved in the data management process.
The ontology helps to understand the complexity of quality of data as well as of images and can be used to describe and manage quality calculation web services in a Service Oriented Architecture.

Stack or Trash? Quality assessment of virtual slides.

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France

Background
At this time, the quality of a Whole Slide Image (WSI) is verified a posteriori by a technician or a pathologist. If a WSI is of insufficient quality, it needs to be scanned again. A high-speed automatic quality assessment of a WSI during or after digitization would therefore greatly improve laboratory workflow.
Although there are multiple ways to assess the quality of a flat image in terms of blurriness, color, brightness and contrast, quality assessment of a WSI is more complex because of its intrinsic structure.
Findings
We describe here a fast method to automatically assess WSI quality and to accept or discard them at the time of acquisition, in less than a minute. A WSI is analyzed at its different magnification levels. Tiles containing no specimen are discarded from the set of images to analyze. Remaining tiles are then analyzed with different tests such as blurriness, contrast, brightness and color. Each tile receives quantitative and qualitative scores for each of the analyzed parameters. Once the tile analysis is completed, parameters are weighted by pertinence and a global score indicates whether the WSI is suitable for further use. This method has been successfully validated through a web survey, showing a strong correlation between our method and visual quality assessment.
Conclusions
This fast method, designed at first to improve laboratory workflow, could also be used as a calibration and quality control tool for a wide range of WSI acquisition systems.

Development of System Resolution Evaluation Slide Set for Whole Slide Imaging: Toward Standardization

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Introduction: System resolution of Whole Slide Imaging (WSI) system is very important toward standardization. While the most common reason for the variations of color and image quality is the variance in the protocols and practices in the histology lab, the image displayed can also be affected by variation in capture parameters, image processing and display factors in the digital systems themselves. It is difficult to identify the exact cause of the problem. We have developed the methodology to evaluate the overall balance as a system and identify the cause of the problem of image quality, color and system resolution. In addition to the two previously developed slide set for color and image quality, we developed another slide to evaluate optics of the WSI system.
Methods: Using the two previously developed calibration slides, the evaluation of image quality and color standardization can be done but the exact cause of the image quality issue in the system can not be identified. The 3rd slide was developed to evaluate the optics quality of whole slide imaging system. These three slides were scanned by several different manufactures' scanner. From the corresponding whole slide images of the two calibration slides we previously developed we evaluated the color characteristics of the scanner and the image quality of the whole slide images each scanner produced. On the other hand, from the scanned image of the new slide, we evaluated the balance between optics, electronics and software and determined the cause of image quality degradation and color variations.
Results: The usefulness of the color and image quality evaluation slides was demonstrated. Furthermore, the new slide for the optics evaluation showed interesting results. Some systems showed very good results in the image quality and color but not for the new slide. After the investigation of the system components, we found that these systems had relatively lower quality of optics compared to the image acquisition device. The well balanced system showed the same level of results by the image quality slide and optics evaluation slide.
Discussion: The importance of the additional slide to evaluate the optics of WSI system from many aspects was demonstrated. The new slide could help to design a well balanced system with minimum cost and also help to improve system resolution toward standardization.

Implementation of telecytology for online quality assurance programs - Georgian experience

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Introduction: The study aimed evaluation of the possibility to use telecytology and clinical information system as tools for implementation of online quality assurance programs under the conditions of Georgia.
Methods: 100 Gynecological cytology cases (benign 52; atypical squamous cells of undetermined significance (ASCUS) 28; low-grade squamous intraepithelial lesion (LSIL) 7; high-grade squamous intraepithelial lesion (HSIL) - 13) were randomly selected. Cases were diagnosed routinely by 3 certified cytologists with an experience of telecytology and usage of clinical information system who provided cytology diagnoses. Digital images were obtained on all cases at a maximum resolution of 2048x1536 pixels and uploaded at clinical information system. The cases were labeled QA. They were reviewed by the same certified cytologists in the frames of pilot online quality assurance program.
Results: Diagnoses were recorded as benign, ASCUS, LSIL, HSIL, and inadequate for diagnosis.
Discussion: Digital images are suitable substitutes for glass slides; telecytology can be used in quality assurance programs; clinical information system can be applied as a platform for online quality assurance programs.

C4 E-SCREENING

Diagnostic reproducibility on whole digital slide in cytology and histology in oncologic screenings

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Background: Diagnostic reproducibility and accuracy in cytology and histology are major issues in Oncologic Screenings (cervix, breast and colorectal cancer). Slides standard set represents the most used method to compare diagnostic proficiency. Emilia-Romagna Region (Italy) realized a software to manage cytological and histological whole-slides digital images building a picture archive and communication system (PACS) among regional pathologists and realizing an e-learning platform (Docebo) for applicants: slides were "teaching objects" for a seminar educational software. Collected and cataloged cases in an on-line digital archive of slides, enact as database for a casistic atlas online.
Findings: Diagnostic reproducibility test has been performed in cervical and colorectal cancer screening, and in breast cancer it is ongoing. In all seminars approx 30 cases have been selected by a committee of Pathologists from regional units. Clinical data were available and all cases were anonymized.
Slides were digitalized using an Aperio scanner and put online for 30 days. The collected diagnoses were matched with the diagnoses stated in a final consensus conference and statistically evaluated by Cohen's Kappa.
Conclusions: Whole digital slide is suitable for proficiency tests and the internet platform allow to share cases and to get the answers of participants better than the circulation of a set of conventional slides.
The quality of whole slides is diagnostic, approaching optical microscopic resolution giving an overall good statistical performance of readers, also in the cytological environment.

Mobile satellite teleoncology

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INTRODUCTION: The Instituto del Cáncer SOLCA Cuenca (Ecuador) for their mission and commitment to the community, based on statistics provided by the Regional Tumor Registry, created in 2010, the PROGRAM OF PREVENTION AND EARLY DIAGNOSIS OF CANCER same as intended to prevent major neoplasms present in our population (Skin Cancer, stomach, prostate, breast and uterus) where together with the implementation of a mobile unit promotes the application of mobile and satellite telecommunications in oncology.
MATERIAL AND METHODS: The project consists of a vehicle adapted for this purpose, called "mobile unit", with offices for: triage in clinical, gastroenterology and endoscopy, gynecology and mammograms, as well as sampling for PSA testing and skin biopsies.
In that unit, at first instance was installed a 3G modem for internet connection through mobile phone network ,that allows the transmission of video data and the institution, and later saw the implementation of a BGAN Broadband device; a portable terminal that enables connection to a global satellite network that allows Internet data transmissions up to 492 kbps in those remote places where it is available GSM network. All these devices have been attached to the home network and computer service in the mobile unit, so that by the free communication software to stream data to the central unit.
With this devices can transmit images to view the cervical cytology, breast images, and clinical images that transmitting data using the software related to our department of pathology in order to clarify diagnoses with relevant specialists and determine immediate clinical behavior, raising the level of efficiency and optimizing resources, saving time at the start of appropriate therapy.
RESULTS: The MOBILE UNIT of the Instituto del Cáncer SOLCA Cuenca attended 12966 in 2 years of work, where we developed and implemented the use of mobile telecommunications creating a new concept of Tele-Oncology, entitled "Mobile Satellite Tele-Oncology ", where with the Internet connection via 3G, and satellite technology enables remote diagnostics from anywhere village located in our province.
CONCLUSIONS: The use of telecommunications tools and resources available have to optimize the overall objective, the prevention of cancer, time, human and financial resources. In addition allowed us to work together both our central and remote unit providing efficiency and effectiveness of diagnosis as a human technology arm of our institution.

Telepathology in cervical and breast cancer screening programmes

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Background
In Veneto, Italy, an organized effective mass-screening programme for cervical and breast cancer has been active. An Aperio ScanScope-XT slide scanner have been applied to EQA since 2009.
Findings
From 2009 to 2011, 1-3 cervical and breast cytologic and histologic samples were selected from routine files in each laboratory participating in the project. The samples have been digitalized by using a ScanScope-XT. The images have been stored on a virtual slide repository available online for a web consultation. The discordant cases have been widely discussed in a year plenary meeting.
23/24 laboratories active in Veneto joined the project. Virtual slides have been created from: 98 cervical smears, 102 corresponding biopsies, 52 breast cancer samples, 48 breast needle core biopsies and 95 breast FNAC. On virtual cervical slides, a total of 1717 cytological and 1719 histopathological diagnoses have been obtained; on virtual breast slides, a total of 1717 cytological and 1822 histopathological diagnoses have been obtained.
In 2009 only 59% of the participant laboratories for cervical EQA and 80% for breast EQA could successfully evaluate the virtual slides. In 2011 the virtual slides have been successfully evaluated by all participants.
Conclusions
The participants agreed that virtual slide is a useful potential modality for EQA. They agreed that major issues limiting the use of virtual slide-based EQA did not involve image acquisition but rather image management issues such as the pathologist's interface or the hospital's network.
These results are encouraging to purse this workgroup, with a very good cost/benefit ratio.

Pap Smear Cell Image Classification Using Global MPEG-7 Descriptors

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Background
Although cervical cancer is fully curable if diagnosis is early achieved, it is still the most frequent female fatal disease worldwide, the second death cause in female population. On the other hand, Papanicolau smear test is the screening method for detecting abnormalities in the uterine cervix cells, including the precancerous cells. Provided that this test is extremely labor intensive and reader dependant, automated screening has been pursued during last decades, resulting in some commercial developments that identify suspicious regions i.e. regions with the possibility of containing malignant cells, but without an accurate identification of pathological cells. As a consequence, cell classification is still an open research problem, usually approached by characterizing morphological features that are highly dependent on the accurate segmentation of the boundaries. In this paper we propose a cell classification method that instead of attempting to segment the cell cytoplasm and nucleus, it characterizes the very inner cell relationships using global features and a standard classifier, the k nearest neighbour, that learns a particular data partition.
Findings
The cell classification approach is carried out using color and texture MPEG-7 descriptors, specifically the color layout, color scalable and Edge Histogram descriptors. The proposed approach raises a mean sensitivity of 84% and specificity between 82% and 89%, which is really promising since no image preprocessing was carried out.
Conclusions
The most relevant feature was the edge histogram, with which the best results were reported, whilst combination of this feature with the color scalable feature reported the poorer performance. On the other hand, modification of the learning parameters, did not significantly change results w.r.t. the classification task.

A9 TECHNOLOGICAL ADVANCES: LABEL-FREE TECHNOLOGIES

Raman micro-spectroscopy and multi-spectral imaging for Lymphocyte classification

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Background
Current diagnostic and prognostic approaches in oncology are based on morphological and molecular techniques which are still complex and hard to standardize. This is also true for Chronic Lymphocyte Leukemia that has been chosen for the IHMO project. It is a blood cancerous disease characterized by the proliferation of lymphocytes and generally shows no clinical signs and thus is often discovered by chance during a blood test. IHMO project aims at simplifying tumor diagnosis and prognosis by developing a multimodal microscopy platform that includes in a single machine Raman micro spectroscopy and multispectral imaging.
Findings
Blood smears were prepared on microscopy slides; cells were localized by optical microscopy, and Raman micro-spectroscopy spectra were acquired on cell nuclei. After staining, ten bands (transmitted visible light) multi-Z stacks images of each cell were acquired. Raman molecular signature were classified using a Support Vector Machine algorithm that allowed for distinguishing lymphocytes from other nucleated blood components with 99.6% sensibility and 98.8% specificity. Then the algorithm was used for developing a classification model splitting leukemic and healthy smears with 80% sensitivity and 100% specificity.
Morphological descriptors obtained from multi-Z and multispectral images provide another independent classification that still needs to be assessed.
Conclusions
The results obtained in this study have shown the potential of such a multimodal approach. Moreover, the developed multimodal microscopy platform can be used more generally in the field of cyto-hematology, or parasitology. However application to cytological and histological pathology would need further developments and could take profit from new methods in data classification.

Highlighting peritumoral areas in human skin cancer biopsies by infrared micro-spectroscopy

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France

Background
Infrared spectral imaging coupled with clustering methods has been shown to be efficient to highlight the structural composition of human tissues and to provide clinically relevant diagnostic information for oncology. Especially, a new fuzzy C-means based algorithm was developed in order to automatically process an infrared image and to assess the transitions between structures (e.g. tumor/normal) in a tumor tissue. However, this algorithm is time consuming and the visualization of the transitions is not easy.
Findings
An upgraded version of the fuzzy C-means based algorithm has been developed in order to overcome these problems. To reduce the computational time, the fuzzy C-means algorithm has been modified and the number of computed fuzzy C-means reduced. An entropy based interconnectivity measure was defined in order to quantify the overlapping between fuzzy clusters and thus to ease the visualization of the transition areas. Applied on a squamous cell carcinoma, the new algorithm was able to retrieve the tissue structure and to highlight the existence of an invasive front. Furthermore, the interconnectivity measure showed that the tumor is highly connected to the invasive front which is surprisingly completely dissociated from the surrounding dermis.
Conclusions
IR spectral microimaging associated with clustering techniques shows a great potential for probing tumor progression and for early determination of tumor aggressiveness in cutaneous cancers.

Infrared spectral imaging applied to paraffinized tissue microarrays for colon cancer diagnosis

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France

Background: Colorectal cancers are the third most common type of cancers globally affecting both sexes (1). As of now, histopathology is the gold standard method for colon cancer diagnosis. Newer technologies are the important need of the hour to complement the existing approaches, for better understanding the onset and progression of the disease. Infrared (IR) imaging could be a potential candidate method because of its capability to probe non-destructively and in a label-free manner the intrinsic chemical bonds present in the tissue, thus giving a "spectral fingerprint" of its composition and structures. To this end, we have developed IR spectral histopathology with the aims to: (a) examine, the molecular changes between normal and tumoral colon tissues, (b) exploit its potentials to identify new diagnostic markers to complement conventional histopathology, and (c) develop an algorithm as an automatic diagnostic tool for tumor prediction, directly on paraffinized samples, without chemical de-waxing, staining or any further preparation.
Material and methods: Nine normal and 25 tumoral colon tissue sections (3mm diameter x 10 μm thick) embedded in the form of paraffinized tissue microarray (TMA), stabilized in an agarose matrix were analyzed directly by IR imaging. To avoid chemical deparaffinization, a modified Extended multiplicative signal correction (EMSC) method (2) was used to digitally neutralize the spectral interferences of paraffin and agarose, and to preserve only the biological information from the tissue. Corrected spectra were classified using k-means to construct color-coded images using the hematoxylin, phloxine and saffron (HPS) stained sections as reference. Linear discriminant analysis (LDA) was then used to construct a prediction model for identification of blind samples.
Results and discussion: EMSC permitted mathematical correction of the spectral interferences originating from paraffin and agarose. K-means classification allowed to identify and to distinguish important histological features of the colonic tissues such as crypts, lamina propria, tumor, etc. When compared to HPS stained images, after whole slide image analysis with crop and score Calopix module from TRIBVN or through pathologist control, color-coded spectral images not only reveal features representative of the biochemical make up of the tissues, but also highlight additional features like intra-tumoral heterogeneity and tumor-associated stroma, which are difficult to discern by conventional histopathology. The LDA prediction model was promising since an average sensitivity of 91 % was achieved in the identification and prediction of tumoral tissues.
Conclusion: IR imaging allowed differentiating and detecting normal and tumoral colon tissue features based on their intrinsic biochemical information. This chemical-free approach on paraffinized tissue biopsies combined with multivariate statistical image analysis opens a new avenue for numerical spectral histopathology and appears as a promising tool for colon cancer diagnosis. Further work to improve the model and to predict tumors in blind samples is ongoing.

B5 IMAGING TECHNOLOGY

Color correction and enhancement for histopathology images

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Introduction
Staining-color correction for hematoxylin and eosin (H&E) stained histopathology images has been investigated. Staining errors are not only evident in H&E stained images but also in special stained images. Errors in staining could affect the differentiation of tissue structures. It could also affect the outcome of a diagnosis, especially when the errors encompassed the object of interest. In this present work we investigate the possibility of correcting the staining errors in histopathology images while at the same time providing enhancement to the image.
Methods
A multispectral image acquisition device whose spectral sensitivity is within the visible spectrum, i.e. 410nm-720nm was used to capture the special stained histopathology images. The N-band spectral samples of the different tissue components found in the images were collected. Two NxN matrices were formed using the spectral data: (i) the first matrix is to correct the staining color of the background structures; (ii) and the second matrix is to correct the staining color the structure which is identified by the user to be the object of interest. The first matrix is determined by least square method using reference and target spectral samples, while the second matrix is designed depending on the staining color that the user desired to be impressed on the object of interest. The original spectrum of a multispectral pixel is scaled by the elements of the first matrix and then shifted by the product between the elements of the second matrix and the pixels spectral residual-error. The spectral residual-error is the difference between the original spectrum of the pixel and its reconstructed spectrum using M<<N principal component vectors.
Results
Multispectral images were captured from Massons trichrome and Hematoxylin and eosin (H&E) stained tissue sections. The captured images showed varying staining color attributes. Spectral samples of nucleus, cytoplasm, red blood cells (RBC), and that of the white areas (the areas which do not contain any tissue structures) were collected. Using the spectral data of these tissue components the two NxN matrices were determined. Results of our initial experiments show the potential of the approach to correct the staining colors of histopathology images while at the same time enhancing the visualization of the tissue structures.
Discussion
Stained tissue structures displays unique staining patterns or colorimetric attributes which pathologists are accustomed to. Variations from these patterns might affect their visual perceptions and in turn influence the outcome of their diagnosis. Staining error correction could be beneficial in providing pathologists the images they are most familiar with. Moreover, being in the era of digital pathology wherein we are leaning towards incorporating digital image analysis in pathology diagnosis workflow the digital implementation of staining error correction would likely increase the accuracy of the analysis.

Towards the integration of digital cytology in the tablet technologies

Maria Rosaria Giovagnoli, Marco Pochini, Mauro Grigioni, Enrico Giarnieri, Daniele Giansanti
Italy

Background: The study is focused on the technological aspects of Digital-Cytology approaching a new scenario relevant to the navigation and cooperative decision. The today' s technology allows an easy way to share information relevant to image by means of different tools. The tablet technologies are the more promising ones available to share image-information in Digital Cytology.
Findings: The first part of the study reviewed the tablet technologies. They have been grouped in three categories:
a) Wearable tablets such as the Smart-phones.
b) Portable tablets such as the A4 and A4/2-Tablet as for example the Apple Ipad.
3) Not portable tablets such as the Xdesk by Epson.
The first two systems are today widely used in many applications. As these two systems allow to reach everyone in the World thus represent a chance for the remote consulting in Digital Cytology.
The Epson XDesk has been investigated as the third system. This system comprehends an interactive table with a 52-inch screen and a 1024x768 touch screen display with all the tablet functions. The second part of the study

focused to the design of a HTA tool based on an interactive form to investigate the different technologies in performance and in acceptance.
Conclusions: The Tablet technologies have been reviewed and a HTA specific tool has been proposed. Possibilities and limitations of the three different technologies will be deeply investigated by means of the proposed HTA methodology on experts and students approaching the new scenario of digital-cytology

A Service Oriented Multilayer Architecture for Virtual Microscopy in Mobile Devices

Luis Martínez, Germán Corredor, Marcela Iregui, Eduardo Romero
Colombia

Background
Mobile devices have undergone a large transformation in the last decade, increasing their use and their possibilities. In the last two years, modern tablets have been used in many different applications because of their computational and display capacities. The possibility of using such devices in virtual microscopy highly depends on robust models that allow interacting with virtual slides. This article introduces a service oriented architecture that optimizes data interaction by a multilayer implementation of the well known JPEG2000 standard.
Findings
The presented architecture is composed of four layers. The first layer is charged of managing the data storage: the compressed images and the index files. These index files store the compressed image structure and the distribution of packets in terms of JPEG200 granularity, i.e., different component, resolution, quality and spatial region. The second layer is responsible of interacting with the data, dealing with different requests. This layer optimizes the access to the compressed file and rules the exchange of information by storing packets with large probability of use. The third layer, the proxy, handles connections with multiple clients and maintains those services associated to wavelet computation and to decoding and dequantizing operations. Finally the client layer reconstructs the image from the wavelet coefficients available at the proxy layer.
Conclusions
The proposed architecture was evaluated on mobile devices, presenting good performance with different resolution levels and quality layers. Likewise, reconstruction times were of the order of hundred milliseconds for low resolutions.

An Image Repository for Decision Support

Ole Eichorn

USA

Pathology is a visual field, and pathologists make decisions based on visual information. An online repository of easily searched, well-categorized pathology images can be of immense use to pathologists, scientists, educators, and students as a reference.
Individuals and institutions all over the world have accumulated collections with thousands of slides representing a huge variety of pathologies. Some collections are already digitized and available online, while others remain libraries of glass slides, with a large variety of formats for case information and other metadata. Working with collectors to publish their slides online and aggregating them together can yield an amazing decision support resource.
Long-term archival storage is a key issue for organizations adopting ePathology. A cloud-based repository of images can provide an inexpensive and scalable solution.
ePathology is increasingly being used for remote consultations. The cases managed by online consultation networks can be de-identified and contributed to a common repository, increasing the value of the decision support tool while providing a long-term archive.
This talk will present concepts for an Image Repository for Decision Support, and discuss some of the technical and business considerations for making it a reality, including ways to curate and enhance image metadata, organizational principles and search tools, relationships with users and contributors, access methods, and security and privacy considerations.

A10 IMAGE ANALYSIS

Overcoming Challenges in Histology 3D Imaging

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USA

Introduction:
WSI technologies and rendering software have improved to the point that 3D reconstruction of large structures at microscopic scale from hundreds of serial sections became possible. 3D Imaging has the potential to bring about new discoveries in medicine. However, challenges remained such as section registration, quality of tissue and the effects of tissue processing and sectioning all must be optimized, and the huge amount of data that can be generated must be processed, stored and made available as quickly and efficiently as possible although we have been working on overcoming these issues for last several years, it was not easy and had limited of usage of 3D imaging.
Very recently, we improved the quality of consecutive image alignment technology and speed of reconstruction. It enhances the value of histology 3D imaging and opens up more possibilities.
Methods:
Specimens included mouse embryo, kidney, lung and stomach 50-250 serial sections were cut manually or by an automated sectioning machine (AS-200, KURABO INDUSTRIES LTD. Japan) from formalin-fixed paraffin-embedded blocks and stained with H&E. Serial sections were scanned at 0.33um/pixel using a Mirax Scan device (3DHISTECH Ltd, Hungary). 3D reconstruction was done using the algorithms developed by co-authors. To improve the quality of consecutive image alignment, new algorithms incorporated intensity values into the registration process underlying the image reconstruction. To this end, similarity measures working on pixel color values instead of extracted landmarks drive the iterative algorithm, which optimizes the relative geometric location between neighboring sections dramatically. To improve the reconstruction speed, new algorithm used a combination of image pyramids and region processing: Image stacks were initially reconstructed on a low magnification level, which did not cause too much computational workload. If a user selects a region of interest by zooming into the initially reconstructed volume, only the alignment of this particular region is updated discarding surrounding regions.
Results:
All 3D reconstruction results were improved from previous version. Previously when a case contains over 100 slides, we had to divide into multiple models due to the computer capacity. This time, 150-200 slides were reconstructed at once with the same computer. The improvement of the quality of consecutive image alignment gave us the opportunity to segmentation of region of interest and measure the volume and size of particular tissue components or organ and segmentation.
Previously, we spent several hours to a couple of days to reconstruct one well aligned 3D model. However, with new technique it took 3-5 minutes for initial reconstruction and another 10 minutes for detailed reconstruction. Many usages of 3D imaging such as radiology view and blood vessel segmentation will be presented at the conference.
Discussion:
We believe that the technology for histology 3D imaging finally reached the level we expected many years ago. Next step is to improve the method to see the correlation between the 3D reconstructed images of histology slides with other 3D imaging modalities such as the microCT and optical frequency domain imaging (OFDI). The interface between different 3D imaging modalities has the potential to bring about new discoveries in Medicine
Acknowledgement:
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An entropy-based automated approach to prostate biopsy ROI segmentation

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Spain

Background
Whole slide imaging (WSI) devices, i.e. robotic microscopes and scanners, provide the starting point for computer-based processing of microscopy image, providing a complete toolkit for computer-aided diagnosis (CAD). This include biopsy analysis, which is crucial to make an accurate diagnosis in some diseases such as

prostate cancer. A fully automated approach to region of interest (ROI) segmentation in prostate biopsy images is proposed here.
Findings
The developed algorithm is based on local entropy and YIQ colour model analysis. The automatic segmentation of ROI would permit the pathologists to focus on the most important areas of the image.
Conclusions
The algorithm is capable of dealing with full WSI biopsies digitized at 10x magnification, whose typical sizes take from 150 up to 300 MB. The tests carried show that the algorithm is fast and accurate. The proposed algorithm is also original because it works on large images acquired with low magnification, thus being different from other algorithms that require higher magnification and have been tested only on small samples. In this way, the method tries to mimic the manual procedure of expert clinicians.

Cell Nuclei Extraction from Breast Cancer Histopathology Images Using Color, Texture, Scale and Shape Information

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Singapore

Background: Cell nuclei extraction from Haematoxylin and Eosin (H&E) stained breast cancer slide images is a challenging task due to the high content complexity of images: nuclei have heterogeneous appearance and overlap while the background is complex and non-homogeneous. This causes standard extraction methods to perform poorly.
Findings: In this paper, we propose a machine learning based method for obtaining a new image modality based on colour, texture and scale information, which allows to significantly improve the accuracy of nuclei extraction. First, colour deconvolution is performed to separate Haematoxilin and Eosin from which 45 rotation invariant local texture features based on Laws' texture energy measures are computed. The features are computed for every pixel at 4 different scales obtained by image re-sampling using Lanczos-3 filters. The obtained 180 features are then passed to a classifier able to predict the probabilities of each pixel to belong to a nuclei or the background. The classifier combines Linear Discriminant Analysis (LDA) followed by a softmax transform. The resulting probability map presents both homogeneous well-differentiable nuclei and background. Finally, nuclei are extracted using an Active Contour (AC) model which includes shape prior to deal with overlaps.
Our approach is based on supervised learning to train the classifier and to determine the parameters of the AC model. Therefore, it is easily adaptable to other types of objects or image modalities. The training set is constructed using images in which nuclei have been manually delineated by a pathologist.
Conclusions: An empirical study shows that our method significantly improves the quality of nuclei extraction, specially for high histological grade cancers with pronounced cytonuclear atypia.

A11 DIGITAL IHC 2

Comparison of image analysis of IHC, visual scoring, and quantitative real time PCR data for Ki67 tumor proliferation in breast cancer

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Germany

Introduction: The exact determination of tumor proliferation is of outmost importance for clinical decision making in breast cancer. According to the 12th St Gallen Guidelines systemic therapy, the luminal subtypes are stratified according to Ki67.
Methods: Tumor samples from 90 patients that participated in a neoadjuvant clinical trial were aggregated on a tissue microarray using 0.8 mm cores from the center and from the tumor periphery. Immunohistochemistry was performed for the Ki67 antigen on an automated IHC platform (Dako Techmate 500). Tumor proliferation was assessed after segmenting the TMA cores with TMA-Lab II (Aperio) using the Aperio Image Analysis toolbox. For this purpose, a tumor-stroma segmentation classifier was developed with the Genie tool (Aperio), and applied to the Ki67 stained cores. After manual correction of the segmentation results, the Ki67 staining index was assessed be the IHC nuclear tool (Aperio) in the tumor cells. The results were compared with RT-qPCR data that were obtained after extracting RNA using Qiagen kits. RNA was isolated from fixed tissue samples by using coated magnetic particles.Multiplex RT-qPCR was performed by TaqMan® based primer probe sets for Ki67, and CALM2 as reference gene. Single Step RT-qPCR was performed by using Invitrogen reagents on a Stratagene MX3005p.
Results: The Genie classifier was correctly identified the tumor cells in > 90% of cases. Statistically, we have observed a significant correlation of mRNA data from independent, matched fresh and fixed biopsy samples by using RT-qPCR (ER1 r=0,91; PR r=0,79, Ki67 r=0,72). When comparing automated image analysis results with conventional histological evaluation of IHC markers, there were only 3 cases misclassified for ER positivity or negativity, and 4 cases misclassified for PR positivity or negativity. Correlation of RT-qPCR data with quantitative immunohistology was highly significant ($p<0.0001$). Similar results were obtained for RG-qPCR of FFPE tissue.
Discussion: Image analysis can be sucessfully applied for the determination of the Ki67 tumor proliferation index by using a tumor-stroma segmentation classifier and a nuclear analysis tool. The determination of Ki67 mRNA analysis of fresh and fixed tissue samples results in robust and comparable results when compared with quantitative immunohistochemistry. Both methods are more accurate than conventional visual analysis of IHC staining.

Impact of tumor heterogeneity on disease-free survival in a series of 368 patients treated for a breast cancer

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France

Background
Tumor heterogeneity is an old concept but its impact on cancerogenesis process is poorly understood. Breast cancer is a noteworthy model for its frequency, the diversity of its phenotypes and of its evolution. This study investigates the influence of the heterogeneity of tumor proliferation on disease-free survival of patients with a breast carcinoma. The study involved a series of 368 patients from the François Baclesse Cancer Centre (Caen) with a follow-up of more than 15 years.
Findings
An immunostaining of mitotic figures was performed on histological section, representative of the tumor, with the anti-phosphohistone-H3 antibody (PHH3). The slides were scanned, then sub-sampled and analyzed by an automatic image processing program developed by the lab. This program detects “Hot Spot” areas and measures 9 features of heterogeneity.
A principal component analysis was used to extract the most relevant features for heterogeneity (4 were selected) and combine them (CP2). They were analyzed statistically. In the univariate analysis (DFS), only the Voronoï feature and the combination CP2 have a prognostic value. The multivariate analysis (Cox), combining with

classical clinicopathological factors and heterogeneity features, showed three independent prognostic factors: Tumor heterogeneity (CP2), Mitotic index and Lymph node metastasis.
Conclusions
The construction of this model has individualized three groups of patients: “0”, “1 or 2” and “3 poor prognostic factors”. The tumor heterogeneity measured by image analysis is one of the three major prognostic factors identified in our series. This result encourages confronting the heterogeneity feature CP2 to clinic information, such as recent or late oncologic event.

The breast cancer, the Her-2/neu gene, the pathology lab and the digital pathology: time to revise the workstation of a modern pathologist and perform a molecular diagnosis in front of a modern widescreen display, near our friendly microscope.

Matteo Brunelli, Isabella Daniele, Serena Pedron, Stefano Gobbo, Diego Segala, Guido Martignoni, Marco Chilosi
Italy

The breast cancer, the Her-2/neu gene, the pathology lab and the digital pathology: time to revise the workstation of a modern pathologist and perform a molecular diagnosis in front of a modern widescreen display, near our friendly microscope.
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INTRODUCTION
The instruments in the digital pathology unit have/are going to modify/ied the workflow of labs, enhanced the pathologists role in diagnostics. Digital image analysis is actually available in a variety of platforms to improve performance of diagnostic molecular pathology. Worldwide, automation of the fluorescent in situ hybridization technique (FISH analysis) is under consideration. Clinically, the determination of the Her-2/neu gene amplification is of importance, as 20-25% of patients affected by invasive breast carcinomas come along with HER2/neu overexpression, and a targeted therapy is available for treatment in this setting of patients improving outcomes.
We sought to evaluate the performance in detection of the Her-2/neu gene status in a serie of breast carcinomas by a digital microscopy using the slide scanning-platform D-Sight Fluo.
METHODS
30 infiltrative breast carcinomas (grouping three clustered common set, amplified vs not-amplified vs equivocal) were scored either automatically by scanner D-Sight/Fluo and visually by a specialist in the field using a routinely available Olympus BX61 fluorescent microscope. Virtual slides were available and the score numbers were compared. The D-SightFluo automatically calculated the HER-2/CEPT 17 ratio both for single nuclei and globally for the set of images analysed. A gallery of all nuclei arranged by score were visualized.
RESULTS
11/30 cases showed gene amplification at visual level by using the routinely Olympus DX61 instrument; 9/11 (81%) matched with the ratio automatically scored on digitalized and scanned slides by the D-Sight Fluo instrument. The 2 single discordant cases showed heterogeneous/low level borderline Her-2/neu gene amplification. Possibility to view all focal plane images as an image stack for FITC and TRITC and the possibility to edit nuclei and spots permitted to re-score the cases appropriately. 18/30 (19%) showed a not amplified pattern and 16/18 (89%) matched blinded. 2/18 (11%) showed monosomy of chromosome 17 or heterogeneity.
DISCUSSION
The automation of Her-2/neu fluorescent analysis by D-Sight Fluo instrument in breast carcinoma show a good performance and may help pathologists in screening the Her-2/neu molecular gene status; 2) D-Sight Fluo may support the networking and e-learning on FISH medical training; 3) the digital FISH images may be stored, overcoming the fading of the fluorescent signals; 4) the workstation of a pathologist may be revised and actually a pathologist may perform a molecular diagnosis in front of a screen desktop rather than in front of a microscope or in front of a modern screen desktop near our friendly microscope.

B6 E-LEARNING

Teachers´ impact on dental students´ exam scores in teaching oral pathology using digitized slides

Janusz Szymas, Mikael Lundin, Johan Lundin
Poland

Background
We have developed and evaluated a user-friendly on-line interactive teaching and examination system for Pathology of the Oral Cavity. Abandoning the use of conventional training microscopes and glass slides since 2005, we decided to rely on virtual microscopy to facilitate learning of oral cavity pathology to dental students. Because of a high number of new students enrolling every academic year, students work in groups supervised by different teaching assistants with heterogeneous teaching experience. The aim of this study was determining whether in this context the teacher has still an impact on students' exam scores.
Methods
In the academic years 2005-2006 through 2011-2012 we examined almost 500 dental students. Student groups were supervised by 18 various teaching assistants. Student groups were randomly allocated to teaching assistants. We obtained information on students, including enrollment history and test scores from the administrative records. In this study, we provide descriptive statistics and t-test analysis.
Results
This study provides evidence that the teachers affect dental students' exam scores. The students groups supervised by some teachers obtained better final exam results in comparison to those obtained by the other groups, and this was consistent throughout all the years studied. Students assigned to such high-value teachers were more likely to obtain the highest exam scores.
Conclusion
We conclude that, despite widely available self-study possibilities, good teachers still create a substantial value. Exam scores are helpful in identifying such teachers. This study also shows evidence that existing measures are informative about the teachers' impact on the students' results.

E-education for medical Students using WSI in Egypt

Essam Ayad
Egypt

Background: Classic education of pathology for Medical students in emerging countries with limited resources faces many obstacles because of equipment cost and small laboratories which are not suitable for the large number of the students. Digital Pathology may provide ideal solutions.
Methods: We scanned the whole set of the slides used for teaching practical pathology sessions for third year medical students using the Whole Slide Image [WSI] technique. We upload all this digital material on the computer network in the pathology department or the Grand Student Library in the Faculty of Medicine, Cairo University. A group of our medical students viewed digital pathology slides either in the pathology department or the library using the computer network or their iPad tablet device. They were allowed to view it at home through connecting the server at the Grand Student Library in the Faculty of Medicine, Cairo University. We reported student experience with virtual slides on a local network and a remote image server. Furthermore we compared the results of the digital exam with the classic exam [using glass slides & microscope]
Results: The quality of images of the scanned slides was very good. Comparing the different ways for viewing the slides, we found the best method was using the computer network in the computer lab in the pathology or in the Grand Student Library, it was evidently faster and preferred by the participants in this study, followed by using the iPad tablet device in the library then viewing it at home through accessing the server at the Grand Student Library. The grades of the students using the virtual slides beside the glass slides were much higher than those using the glass slides & microscope only.
Conclusions: Using the WSI Virtual slides for Medical Students learning can be the best solution for equipment and technical obstacles and could enhance student learning in emerging countries with limited resources.

More Technology, Better Learning Resources, Better Learning?

Laura Helle, Markus Nivala, Pauliina Kronqvist
Finland

Introduction
The adoption of virtual microscopy in 2008 created a unique real-world laboratory for exploring ways of reforming the learning environment. The purpose of the present study was to evaluate the impact of a set of measures designed to boost understanding of abnormal histology through an emphasis on knowledge of normal cells and tissues.
Method
The experimental group (n2010 = 20) was exposed to the following set of measures: (1) digital resources to review normal structures and an entrance exam for enforcement, (2) digital course slides highlighting normal and abnormal tissues, and (3) self-diagnostic quizzes. The performance of historical controls (n2007 = 61), who had not been exposed to any of the measures, served as a baseline. Understanding of normal histology (Test A) was assessed in the beginning of the module to determine the impact of the first set of measures, and understanding of abnormal histology (Test B) was assessed at the end of the module to determine the impact of the whole set of measures. Additionally, four students were interviewed.
Results
Results confirmed that the experimental group significantly outperformed the historical controls (by 46%) on Test A. Students reported that the idea of emphasizing normal structures was great. However, on Test B, the historical controls outperformed the experimental group (by 32%).
Discussion
To conclude, allowing students access to high-quality digitized materials and boosting prerequisite skills are clearly not sufficient for boosting final competence. Instead, the solution may lie in making students externally accountable for their learning throughout their training.

Application and evaluation of teaching practical histology with the use of virtual microscopy.

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Background
Virtual slides provide many benefits over observation of classical glass histology slides. We have developed an electronic teaching system of histology practical in a networked classroom of 30 student's computers and one teacher's. The teacher's PC (personal computer) runs our own database of histology practical designed in MS Excel format that contains virtual slides organized into thematic sessions. The virtual slides are complemented with supporting documents and other explanatory visuals. A PC-based testing of identification of structures in histology slides has also been introduced.
Findings
Majority of students of General Medicine and Dentistry evaluated positively the use of virtual slides, as they allowed them to study and also to discuss various details of cells and tissues at various magnifications easily. They also benefited from using the attached supporting documents during practical sessions and self-studies. Regarding PC-based examination, most of the exposed students preferred practical examination in MCQ (multiple choice questions) format over the classical oral examination.
Conclusions
The e-learning format of histology practical based on virtual slides proves to be a didactically efficient method of teaching histology to medical students.
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C5 IMAGE ANALYSIS 2

A morphometric tool applied to angiogenesis research based on vessel segmentation

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Spain

Background
Given that angiogenesis and lymphangiogenesis are strongly related to prognosis in neoplastic and other pathologies, and that some existing methods for their study provide different results, we aim to construct a morphometric tool to allow complete and accurate quantification and measurement of different aspects of the shape and size of vascular vessels.
Findings
The developed tool herein presented is based on vessel closing which is an essential property to properly count the number and characterize the size and the shape of blood and lymphatic vessels.
Conclusions
The method is fast and accurate improving existing tools for angiogenesis and lymphangiogenesis analysis. The tool also improves the accuracy of vascular density measurements, since unconnected endothelial parts are joined and considered as a single object forming a vessel.

Automated classification of breast cancer images according to morphological features using LPQ/LBP texture descriptors and an SVM classifier.

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Finland

Introduction: Grading of tumors is regarded as an unreliable prognostic factor due to its poor reproducibility and variances. Computational diagnostic tools for estimating the morphological properties of cancer tissue would enable objective and reproducible judgment. This could be achieved by fully utilizing recent advances in digital microscopy and computer vision. We propose a texture based algorithm for automated classification of breast cancer morphology. The method utilizes local phase quantization (LPQ) as well as local binary pattern (LBP) descriptors as input data to a support vector machine (SVM) classifier.
Methods: The image data set (n=1092) was extracted from a series of digitized, whole-slide tissue microarray (TMA) samples from a nationwide cohort of breast cancer patients (The FinProg Series). A single continuous area that contain tumor tissue only was defined in each representative tissue spot in the haematoxylin eosin stained TMA samples. The images were scored by a human observer into three classes according to morphology: 1 (morphology resembling normal breast epithelium, extensive tubular formation; n=182), 2 (intermediate tubular formation, n=494), and 3 (morphology least resembling normal breast epithelium, no tubular formation; n=416). The images were transformed to gray scale and represented by LBP and LPQ texture descriptors. Three one-against-all SVM classifiers with a radial basis function kernel were constructed to separate one of class at a time from the others (i.e. 1 vs. 2-3, 2 vs. 1, 3 and 3 vs. 1-2). The data was split in two equally sized parts for training and testing of the algorithm.
Results: The experiments were performed using different combinations of LBP and LPQ variants as well as by various scaling of the images. The best classification results were achieved by concatenating the basic versions of LPQ and LBP descriptors (eight sample points at radius one) into the same 512-dimensional feature vector and using the original image scale 1:1. The AUCs for the ROC curves were: class 1 vs 2-3: 81 %; class 2 vs 1,3: 63 %; and for class 3 vs 1-2: 81 %. If each image is classified into the class with the highest SVM score the total accuracy is 60.0 %. The result was improved by 2 % when using the LPQ descriptor in addition to the LBP descriptor.
Discussion: In the current study we show that a computer vision method based on texture features and a machine learning method can discriminate between cancer morphology as determined by a human observer. It was shown that by combining the LBP and LPQ features it is possible to improve the discrimination accuracy compared to using only LBP alone.

Automatic measurement of the evolutionary process dynamics of primary biliary cirrhosis (PBC)

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Italy

Background. Primary biliary cirrhosis, is an autoimmune disease, semiquantitatively described by four stages obtained from evaluating the stage of hepatic structures. The followng pathologic events, inflammation, destruction-regeneration of biliary tissue CK7+ and fibrosis determine this disease.
Findings.The static (staging) and dynamic grading of primary biliary cirrhosis was evaluated by histological examination of liver biopsy specimen with a new rapid automated technique that gives metrical measures of liver histological elements with a new advanced histological metric gauge we named Histological Metrizer.
The new method, 1) quantizes the three primary parameters which determine the patterns of primary biliary cirrhosis (inflammation, CK7+ biliary network, and fibrosis) directly on the bioptic liver tissue fragment; 2) expresses with scalars the minor tissue structural elements that are invisible by light microscopy; 3) discriminates between small variations of these variables. With these magnitudes we drew the three-parameter metrical "state portrait" of the histological section. Only the scalars of biliary duct fragments and fibrosis islets, were transformed into vectorial values and summed. The resulting dot-like geometrical figure was considered as newtonian particola and taken as dynamic index. The cumulative curve of this dynamic index drawn from the enlisted cases furnished the ideal trajectory describing the dynamic changes in PBC process from α to ω. Three phases were identified on this curve that geometrically represents the primary biliary process.
Conclusion: Quantitative liver biopsy evaluations that reflect simultaneously the stage and dynamic changes of the PBC process were rapidly, precisely, and reproducibly elaborated.

Correlations between intratumoral stromal constituents and tumoral arhitecture in prostatic adenocarcinoma

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Romania

Introduction. The authors made a preliminary assessement of possible correlations between the amount of intratumoral stromal fibrillary components (IFC-ratio), and density of intratumoral capillary network (ICN-D) and the architectural tumoral patterns described by Gleason.
Methods. The studied material consisted of samples obtained by transurethral resection from 34 patients diagnosed with prostatic adenocarcinoma. Prostatic tissue was fixed in buffered formalin, included in paraffin wax and stained, on serial sections, with Hematoxilin Eosin, Gömöri (for IFC) and antibodies anti CD34 (for intratumoral capillaries. Dominant and secondary Gleason patterns were identified for each case. Ten corresponding fields (5 for dominat and 5 for secondary pattern) with no necrosis were selected randomly for both Gömöri and CD34 serial sections of each case using x20 objective. The selected tumoral areas were acquired and quantitative determinations were performed, after image calibration, with an image analysis softweare. IFC-ratio (expressed in %) and ICN-D (expressed in capillaries/mm2) were determined for each field. The 340 selected fields were subdivided in 5 groups according to Gleason (G) patterns. Data were compared using Chi-square test and Pearsons correlation test.
Results. In G2 group (30 fields) mean IFC-ratio was 21.48+/-9.36 and mean ICN-D was 138.92+/-68.553. In G3 group (110 fiels) mean IFC-ratio was 24.567+/-9.218 and mean ICN-D was 211.09+/-99.64. In G4 group (90 fields) mean IFC-ratio was 21.81+/-9.98 and mean ICN-D was 293.94+/-126.57. In G5 group (119 fields) mean IFC-ratio was 33.07+/-11.76 and mean ICN-D was 248.63+/-131.13. For the patterns with evolution trend to necrosis (EN) the results were: G3C (20 fields)- mean IFC-ratio = 27.19+/-7.69 and mean ICN-D = 252.05+/-103.84; G4A (70 fields)- mean IFC-ratio = 20.06+/-8.48 and mean ICN-D = 245.81+/-75.57; G5A (30 fields) - mean IFC-ratio = 32.27+/-18.86 and mean ICN-D = 134.33+/-91.24. For the so called solid patterns the results were: G3A (50 fields)- mean IFC-ratio = 21.57+/-8.59 and mean ICN-D = 167.1+/-86.09; G3B (450 fields)- mean IFC-ratio = 26.99+/-9.73 and mean ICN-D = 245.6+/-92.83; G4B (70 fields)- mean IFC-ratio = 27.94+/-12.46 and mean ICN-D = 462.42+/-126.03; G5B (80 fields)- mean IFC-ratio = 33.37+/-7.73 and mean ICN-D = 291.49+/-117.67).
Discussion
IFC-ratio increased with Gleason pattern both for the entire group (Chi-square= 78,61 - P = <0,001), but also for EN patterns (Chi-square= 41,69 - P = <0,001) and solid patterns (Chi-square= 48,03 - P = <0,001).
ICN-D increased with Gleason pattern only for the entire group (Chi-square= 66,16 - P = <0,001) and for EN patterns (Chi-square= 40,5 - P = <0,001).

IFC-ratio increased with ICN-D both for the entire group (Pearson r = 0.235 - p< 0.0001) but also for EN patterns (Pearson r = 0.208 p = 0.023) and solid patterns (Pearson r = 0.224 p = 0.002).
These preliminary date show that stromal microenvironment try to adapt to the loss of tumoral differentiation by increasing the amount of fibrillary components and by remodeling its capillary network.

POSTERS

CaseConferencing: telecom resource used for an original approach to on-going teaching through case expertise

Frédérique Capron, Jacques Klossa, Dominique Algalarrondo, Gilles Le Nahour, Jean François Pomerol
France

Introduction
In the pathology field, digital slide conferencing capabilities are obviously a highly necessary functionality when multiple participants need to simultaneously view the same digital slide from multiple, remote locations e.g. for frozen section teleconsultation, second opinion on complex cases or e-learning. In this presentation we present an expertise approach that uses teleconsultation to transfer the knowledge from the expert to requesters. In that context, we will also analyze advantages and perspectives in integrating a general purpose solution to a business specialty (pathology) workflow.
Methods
The idea from the department of Pathology in La Pitié University Hospital center is to take advantage from whole slide image (WSI) teleconsultation to provide an on-going teaching service that will enhance the interpretation level of unspecialized pathologists. Slides are sent to La Pitié for expertise; they are immediately scanned and sent back the same day to the emitter service for storage.
For such application the Orange Multimedia Conference module which is a general purpose piece of software has been integrated to the TRIBVN Calopix™ platform. Calopix™ platform is a dedicated pathology platform which is integrated in the hospital workflow between LIS (Laboratory Information System) and PACS. Multimedia Conference is a web conference service widely used and many thousands conferences are opened daily. To meet the healthcare needs the video module has been enhanced for high image quality, large screen compatibility and exchanges traceability. Such collaboration brings secure data transmission and image compression knowhow to pathology business.
Results
The resulting CaseConferencing module allows recently scanned WSI, i.e. for frozen sections, to be shared instantly without any previous server upload between the conference organizer (expert) and up to 25 participants (case emitters and other interested pathologists). The organizer uses the pathology image workstation that allows classical pan and zoom functions as well as annotation or image analysis tools. Invited participants are informed by email and may participate through the use any web browser on PC or Mac. Among main functionalities, session leadership can be transferred to any participant and real time annotations are automatically stored on the organizer PC.
CaseConferencing guarantees higher quality images transmission and speed for a known transmission channel in addition to ensure security and annotation traceability. Such high quality transmitted image allows clear visualization of very thin features like one pixel thick overlays showing image analysis results. Visualization latency is highly dependent on communication network configuration. It has been generally considered as good enough for WSI sharing and in most cases latency was clearly beyond one second. Such service can be efficiently used as a complement of cooperative asynchronous applications commonly used in the field of telepathology when WSI can be shared through a remote server.
The service is currently used since 2011 by the pathology lab in La Pitié University Hospital in Paris to deliver an expertise service toward private and general hospitals. The purpose is to define guideline describing such expertise services so that it could be extended to other university hospitals.
Discussion
Visualisation and diagnostic clearly allows on screen diagnostic follow up. However higher "fluidity" of pan and scan would be required to achieve the same feeling as when viewing a slide through a microscope. Another demand that will be answered is the publishing of the session report which has been stored on the organizer PC.
The main purpose of this communication was to evaluate how a platform dedicated to pathology could take profit from existing multipurpose information and telecom tools. Such integration proved to be good enough for case conferencing application. The next step would be to apply such concepts to meet another telepathology issue which needs to share expert knowledge. More specifically, in the Cloud Computing context, the idea would be to use the PaaS (Platform as a service) layer to take profit from the stored patient data information in conjunction with consolidated formalized specialist knowledge to drive WSI exploration and to produce automated pre-annotation that will make easier and quicker their on-line consultation.

Enhanced Imaging Workflow for Anatomic Pathology: Implementation of a Knowledge Actor

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France

Introduction
In Pathology, the development of slide scanners offers the possibility to acquire whole slide images (WSI) from specimen stored on glass slides and this is the beginning of a new paradigm in this medical domain. Two DICOM supplements were defined by the DICOM WG26 in order to store and display such big amount of data. These WSI images are mainly used for teaching, for research and for collaborative and cooperative applications. However, automated integration in the hospital healthcare enterprise (IHE) needs much more detailed information for i) integrating the digitalization process into the general diagnostic process in a Pathology Department, in other words, to define the WSI "acquisition modality work list", and ii) integrating efficiently image analysis into the diagnostic process, e.g. to create "evidences" from a WSI.
Methods
A pathologist who reads a slide uses an iterative process which includes exploration and analysis phases, each at varied magnification. In comparison, a slide scanner acquires the whole slide or a pre-defined region of interest. In practice, we know that medical questioning may require more sophisticated possibilities like combining low RGB resolution scan (e.g. 5x to 20x) with higher resolutions and multi-z imaging in some specific region which could be addressed by some slide scanners provided one can build the corresponding modality (ies) work list. The same issues happen for "evidence creation" through an image analysis workflow. Workflow can become even more complex when using a multimodal scanner which adds to the current modalities (RGB bright field and fluorescence) complementary ones like quantitative phase imaging for label free unstained samples and Raman micro-spectroscopy for molecular signature acquisition.
We have 2 choices for solving such issues: either the process is embedded in the scanner software inside a proprietary solution, or the process can be driven by a scenario following the medical needs and assigns to each modality unitary task to be executed.
Results
Let us try to scenarize the fairly simple use case of "Malaria". Following the current guidelines, diagnostics and personalized care aims at parasitemia counting and species identification on red blood cells (RBC): step 1 of the workflow asks for the acquisition of 200 fields at 100x and step 2 asks for individual classification of the retrieved infected RB cells. Such description is medically oriented and needs to be traduced in unitary technical tasks. Clearly, there is a need for some additional knowledge that explains how to select the fields to acquire, how to identify infected RBC and what information should be acquired on each RBC: color, MS images with or without z stack.
Discussion
We are in favor of implementing in the workflow the second solution that avoids proprietary solutions and which could be achieved by adding to the IHE workflow a knowledge actor (KA) that will produce and orchestrate the scenario. KA would ask to the order filler patient and case information's in addition to the diagnostic questions. Based on the corresponding guidelines, the KA could then orchestrate a sequence of unitary work lists which can be executed by the microscopy scanner in conjunction with the evidence creator actor. Such solution would clearly separate medical guidelines from modality embedded software making the workflow more scalable.
Trying to benefit from previous IHE integration, we would suggest implementing such an actor in a way similar to the Treatment Management System (TMS), an information system that manages oncology information and is responsible for the scheduling of radiotherapy activities.

Quantitative phase imaging and Raman micro-spectroscopy applied to Malaria

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France

Background
Malaria is due to parasitism of red blood cells (RBC) by protozoan parasites of the genus Plasmodium. Three main parameters have to be determined for patient treatment: parasite species, the rate of infected blood cells (parasitemia), and development stage. Microscopic observation needs a specialist and is time consuming and automation with slide scanner and image analysis is not straightforward. Therefore we thought that for an automated solution, parasite localization could be easily achieved with quantitative phase imaging and molecular

signature for individual parasite identification and classification could be provided by Raman micro-spectroscopy. This study presents the first stage of proof of concept.
Findings
We analyzed 10 fields of an unstained blood smear infected with falciparum parasite at the comparatively low magnification of 40x (100x immersion with manual microscope on stained smears). Phase image of healthy and infected blood cells showed a clear change in the refraction index which appears lower into the plasmodium than in the red blood cell hemoglobin. This allowed an easy determination of a parasitemia of 4.1% to be compared to 3.5% for the smear label. Raman Spectra recorded on the infected cells have been classified and a sensitivity and specificity of 100% were obtained.
Conclusions
The proposed concept proved to be very efficient on this practical use case thanks to the chosen combination of modalities. However, it remains to bring the proof that the proposed solution also works efficiently for early stage infection characterization and differentiation of parasite species. We are currently working on such complementary studies.

Whole Slide Imaging in Digital Pathology: The Impact of Image Compression

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United Kingdom

Background:
WSI is opening new opportunities for quantitative imaging in pathology including possible improvements in quantitative immunohistochemistry (IHC) for biomarker evaluation. Lossy image compression is a necessity to reduce the large file sizes associated with WSI for archiving, transfer and viewing. Most studies to date have examined the impact of image compression on visual quality. The impact of WSI image compression on computer assisted image analysis and measurement quality, and its implications for applications in diagnosis, prognosis and drug discovery, has not been studied comprehensively. The aim of this study was to determine how increasing degrees of lossy image compression influences; i) WSI image measurements, ii) Nuclear segmentation and iii) changes in relative files sizes
Method:
Using a high resolution uncompressed image of a BRCA1 labelled tissue microarray (TMA) containing 113 tissue cores, a 1024×1024 region was selected within each core image. These regions were compressed into new image sets, using JPEG (Independent JPEG Group (IJG)) and JPEG2000 (Matrox) compression algorithms. Images with compression quality levels of 1 (lowest quality) to 100 (highest quality) in increments of 1 unit were created for each 1024×1024 image region. Six commonly used quantitative features in IHC image analysis were then measured across the entire set of compression qualities using intensity and RGB (red, green, blue) images, for the first 50 segmented nuclei. These were determined using a nuclear segmentation algorithm on the uncompressed sample. The image segmentation results were also examined as a function of compression quality.
Results:
Most measurements errors in general stabilised at an image compression quality level of around 20. At the highest level of image compression quality when using the Matrox JPEG2000 library, 5% of the nuclei were segmented in error on average with this error rate steadily increasing as image compression quality decreased. The IJG JPEG gave similar if slightly worse results for segmentation that Matrox JPEG2000, however performed much better over a compression quality level of 95 reaching nearly zero errors at the very highest level of quality. The IJG JPEG image compression library gives smaller file sizes for corresponding quality levels than the Matrox JPEG2000 image compression library until above an image quality setting of around 95. In general WSI scanners are set by default to an image compression quality level of 30.
Conclusion:
This study has provided evidence to support the need for; i) development of segmentation protocols that are robust and resilient to image compression ii) development of alternative lossy compression algorithms that reduce their impact on segmentation and measurement and iii) definition of acceptable levels of compression and associated error for diagnostic specific image analysis algorithms.

Content Based Image Retrieval in Digital Pathology

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Digital pathology has enormous potential in both tissue research and primary diagnostic pathology. The number of digitally scanned images produced globally is rapidly increasing, with a single lab having the capacity to generate hundreds of whole slide images a day using a single high throughput scanner. Besides the use for routine review and clinical research, the content of these images provide a valuable clinical resource for cross referencing of clinical cases. It is especially useful in a scenario where difficult or rare case occur and a pathologist/trainee has limited access to other resources for a second opinion. A content based image retrieval (CBIR) approach for digital slides would be highly desirable so that similar cases from a comprehensive digital archive of images (with diagnosis and clinical follow-up) could be retrieved and assist the analysis/diagnosis of the current case in hand. The aim of this study was to develop a CBIR system for digital pathology.
The proposed CBIR system works in the following way: i) An end-user is able to select a region of interest/concern from a candidate digital slide, ii) a robust set of textural and spectral features are calculated on the selected region, iii) this feature vector derived from the user-given image region is then trained to form a Support Vector using one-class Support Vector Machine (SVM) classification. iv) a large set of virtual slides from a database is then queried in the following way:
a) Corresponding feature vectors for every region of the digital slides stored in the database are calculated.
b) Pattern recognition is performed using the previous trained Support Vector and SVM for all feature vectors.
c) The result from SVM, the so called decision value is then used as indication regarding how similar a region of an image in the database is to the candidate user selected region.
d) Using the similarity metric, the top most similar images were retrieved from the archive.
This approach has demonstrated that it is possible to retrieve images and image components on the basis of similarity metrics. Further work needs to be developed to support high throughput analysis and evaluation on large image libraries. The computation complexity of working with such large imagery as well as the associated feature calculation and construction of large support vector models is substantial. However the system presented here can be viewed as many independent operations being carried out on a large set of data. Further work will seek to exploit the parallel nature of the problem to provide a fast, real-time manageable CBIR system.

Application of a specific Image analysis software (Neurite outgrowth image analysis, BD pathway 855 system) to in vitro model of neurodegenerative diseases.

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Italy

Niemann Pick type C (NPC) disease is an autosomal recessive neurodegenerative lysosomal storage disorder, caused by the abnormal function of NPC1 or NPC2 protein. Both proteins are involved in the intracellular trafficking of cholesterol and other lipids. The deficiency of either of them leads to the accumulation of the endocytosed unesterified cholesterol, gangliosides and other glycosphingolipids (GSLs) within the lysosomes.
Clinically, NPC disease presents a highly variable phenotype, with a time of presentiation ranging from fetal to adult age. Despite the recent progress in characterizing the biochemical and genetic defects in NPC disease, the underlying patho-physiological mechanisms of this disorder are not clear and the currently available therapeutic interventions are limited. In particular, the study of the molecular pathways linking the lipid accumulation to the neurodegeneration has been hindered by the limited availability of neuronal models of the disease. Indeed, the possibility to isolate stem cells from accessible cell sources and to commit them to a neuronal lineage could be of great utility in dissecting the molecular pathway leading to the neurodegeneration in NPC patients.
In order to test if highly plastic stem cells can be obtained from skin biopsies, we applied a well established method that allowed us to expand, from different adult human tissues (heart, liver and bone marrow), a population of cells named Multipotent Adult Stem Cells, or MASC, characterized by a high differentiation potential that includes neuroectoderm derivatives.
Cell cultures were successfully grown from skin biopsies of five NPC patients and two healthy donors. As expected, only cultured cells obtained from patients were positive for Filipin staining of free cholesterol, while all cell lines were immunophenotypically similar to MASC and expressed the pluripotent stem cells markers OCT-4. Therefore, to verify if these cell lines share with MASC the ability to differentiate toward a neuronal fate and in order to develop an in vitro model that could be suitable for dissecting the molecular pathway leading to neurodegeneration in each patient, we applied a differentiation protocol able to drive MASCs towards a neural

fate (Beltrami et al., 2007). A multistep method was adopted and differentiation was monitored both by evaluating morphological parameters and revealing the presence of neural markers such as nestin, MAP2, O4 and GFAP. After two weeks of neural induction, a large fraction of cells (≈50%) acquired a neuron like morphology and stained positive for neuronal markers such as nestin and MAP2, suggesting that cells obtained from skin biopsies retained the ability to differentiate toward a neural phenotype. No GFAP, O4 and OCT4 positive cells were detected at the end of differentiation. By using a specific Image analysis software (Neurite outgrowth image analysis, BD pathway 855 system), we measured parameters linked to neurite outgrowth like neurite maximum length and neurite root count. Performing this analysis we found that, once differentiated, cells obtained from patients developed significantly more abundant and longer neurites than the healthy counterparts. Importantly, distortion of neuron shape, ectopic dendrites and meganeurite formation are the main features described in NPC brain cells, thus suggesting that the in vitro differentiation system is, at least in part, able the recapitulate the aberrancies present in vivo.
These results indicate that it is possible to isolate cells with stem cell properties from skin biopsies of NPC-patients and that these cultures retain the ability to differentiate toward a neuronal lineage.
The in vitro model we developed will be useful to evaluate the impact of different mutations on the pathological phenotype and might represent a powerful tool to perform drug screening on cells obtained from NPC patients presenting different genotypes.

An in vitro, image-based platform to evaluate human stem cell senescence.

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Italy

Heart failure is a leading cause of death in the Western World. Nowadays, many Authors agree on the hypothesis that the terminal phase of this syndrome could be viewed as the consequence of the impairment of the cardiac functional reserve.
Several lines of evidence indicate that stem cell senescence exerts a profound impact on the functional impairment observed in different tissues both during aging, and in pathological conditions.
Over the last 5 years, our group has been involved in the isolation, culture, and characterization of cardiac stem cells (CSC) obtained from the hearts of both: cardiac transplantation recipients (CSC-R), suffering from end-stage heart failure, and from healthy donors (D-CSC).
Cells isolated from failing hearts are characterized by a senescent phenotype and an altered function. Specifically, CSC-R showed a reduced telomerase activity, reduced telomere length, markers of telomere dysfunction. In addition, they expressed markers of senescence, such as p21, p16, and γH2AX, and are characterized by a reduced capacity for proliferation and differentiation.
Purpose of this study was to evaluate the effects of drugs known to interfere with pathways, which have been associated with life-span extension or with cell senescence in mammalians, to verify the possibility to attenuate CSC-R senescence.
Specifically, we exposed 5 different CSC-R cell lines to a 3-day treatment with 4 different drugs (Rapamycin, Resveratrol, Metformin, and DETA/NO) and verified the impact of these latter on cell senescence, cell death and cell proliferation. In order to minimize the number of utilized cells and the request of reagents, cell samples were prepared on imaging-compatible 96-multiwell plates, stained by immunofluorescence for senescence-specific markers, acquired by epifluorescence microscope and analyzed by Image-J.
In this way we have established the optimal, non-toxic, dose of drugs. Additionally, we have accumulated evidence of the interference with the processes of cell proliferation (cell number and expression of Ki67), cell senescence (fraction of cells positive for γH2AX, p21 and p16) and cell death (apoptotic and necrotic cell fraction).
We observed a drug-specific effect, with respect to cell death, where Rapamycin increased of about 1.5 fold CSC-R apoptosis ($p=0.002$), whereas Resveratrol showed a protective effect on cell necrosis, reducing it by 50% ($p=0.0001$). Resveratrol and DETA/NO, instead, were associated with a significant reduction by half of cells expressing markers of an activated DNA Damage Response and p21. Last, Rapamycin treatment was associated with a ≈60% reduction in p16 expression ($p<0.05$). All drug-treated cells showed, respect to vehicle, an increase in cell proliferation as assessed by Ki-67 expression.
Pharmacologic treatment of CSC-R seems to reduce senescence processes and improve their functional abilities, opening new perspectives in novel therapeutic approaches to the treatment of human heart failure.

Co-culture assays: an in vitro, image-based platform to evaluate cell migration and growth in soft agar

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Italy

In the study of the cellular interactions co-cultures are an essential tool.
In order to study the reciprocal effects of tumor cells and tumor supporting cells in modifying crucial tumor-related biological properties, such as migration and anchorage independent growth, we took advantage of an image-based platform aimed at defining the specific behavior of each cell type.
Specifically, DiI-labeled A172 cell lines were co-cultured with glioma-derived tumor supporting cell lines for 2 days and then assayed.
Anchorage-independent growth was evaluated by plating 50,000 cells in a 0.25% soft agar solution in 35-mm plates containing a basal layer of 1% agarose and colony formation was analyzed 2 weeks after seeding. We compared the growth in soft-agar of 50,000 DiI-labeled A172 cells, 25,000 DiI-labeled and 25,000 unlabeled glioma-supporting cells and 50,000 unlabeled glioma-supporting cells. For this purpose, a series of optical sections, obtained by scanning along the z-axis the field of interest, were acquired at a 100X magnification, using the Leica DMI-6000B setup, and acquiring both phase-contrast and fluorescence images. Once obtained the sum image, the number of Di-I labeled and Di-I negative colonies with a diameter greater than 60 microns was quantified. For each replicate, a volume of 500µm3 was sampled.
In order to evaluate in vitro cell migration of DiI-labeled A172 cells, co-culture or not in the presence of un-labeld glioma-supporting cells, a scratch assay was performed. In 96-well plates at high confluence, scratches were created utilizing 200μl tips. Phase contrast and fluorescence images of the scratches were acquired at 3-hour intervals, until their complete closing, utilizing Leica DMI6000B. Images were then compared and quantified by ImageJ in order to calculate the rate of cell migration of each cell type in the different culture conditions.
In conclusion, the optimized image-based platform, taking advantage of fluorescence microscope and image-analysis software, allowed us to specifically quantify the respective behavior of different cell types in co-culture.

Circulating exosomes: an integrated flow-cytometric- and Atomic Force Microscopy-based analysis

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Italy

Exosomes are vesicles, 30-100 nm in diameter, that are released into the extracellular environment via the endosomal pathway from a variety of different cells such as tumor cells, dendritic cells, lymphoid cells, mesothelial cells, endothelial cells, or cells from different tissues or organs. For example, several studies have demonstrated that tumor cells emit considerable amounts of exosomes, which can contain growth factors and their receptors, proteases, adhesion molecules, as well as DNA, mRNA, and microRNA (miRs). Importantly, exosomes can reach remote cells through their release into the bloodstream, lymph and other fluids, and transfer their content to other cancer cells or to non-transformed cells. These events may influence tumor invasion, angiogenesis and metastasis. The finding of glioblastoma-derived exosomes that contain RNAs and proteins characteristic of the glioma in serum of glioblastoma patients suggested that blood-based exosomes may be used as diagnostic and prognostic biomarkers.
In our laboratory we tested several protocols to isolate exosomes from human plasma and results were confirmed by atomic force microscopy and scanning electron microscopy. Moreover, using a multiparamteric staining protocol we were able to identify by flow-cytometry the presence of the circulating nano-particles and to give insight into the possible cell of origin in several human pathologies, including cancer, acquired immunodeficiency and autoimmune disorders.

INDUSTRY-SPONSORED SYMPOSIA

A7: DIGITAL SLIDES FOR SURGICAL PATHOLOGY: MITH OR REALITY?

Symposium sponsored by the Platinum Sponsor **Cloud Pathology Group**

Digital Pathology has many applications that have been and are studied and evaluated by research, including telediagnosis, teaching, automated immunohistochemistry and image analysis. These applications typically involve ad-hoc acquisition of slides, which are used only for the specific aim they were acquired for. A crucial application that might enable any others is the transition from a traditionally glass-based main storage of histologic slides to a digital archive in the form of those already available in other medical specialties and in particular radiology, i.e., a PACS (Picture Archiving and Communication System). A Surgical Pathology Laboratory where diagnostic slides are routinely digitized may provide a number of advantages, including: quick access to previous slides; less physical room occupied by glass slides; seamless usage of the above mentioned applications, with a greater possibility of exploiting available data for telemedicine, teaching and research.
However, such a scenario presents a number of possible issues that need to be taken into account for a safe, efficient and cost-effective implementation: reliability, speed, and image quality of the slide scanner, organization of the acquisition phase, involved personnel (technician and IT specialists) and their training, hardware and software features, etc. The complexity of the scenario might prove to be challenging for the typical management of a pathology laboratory, while economic feasibility might demand for larger scale than a single laboratory.
The present session aims at providing practical insights in this scenario, by means of the description of a current experience carried out in Sweden, and an industrial experimentation carried out by Cloud Pathology Group and involving a competitive massive test of slide scanners compared in terms of throughput and diagnostic image quality. Closing remarks on economic and management issues will be provided by the General Manager of an hospital.

C1: DIGITAL IMAGING IN THE MODERN ANATOMY PATHOLOGY WORKFLOW

Symposium sponsored by **NoemaLife**

In recent years the use of digital images became more and more important in several phases of the diagnostic workflow within a modern Pathology Laboratory.
Digital images allow to evaluate the quality of the surgical specimen sent to the laboratory and to store the diagnostic pictures of FISH analysis. On the other hand, the virtual slides allow to share information between pathologists through telepathology networks whenever a second opinion is needed, and make a virtual library available for educational and formative goals.
Today, modern acquisition instruments, the availability of virtually unlimited spaces for storage, the increase of data transmission speed, the high quality achieved by image analysis, and a modern information system for the management of laboratory allow to integrate this wealth of information with the patient's clinical folder, thus opening the way for very compelling scenarios.
In this work we report an overview of the needs of a modern pathologist, showing a real successful experience achieved at Rovigo ULSS18 with the aid of NoemaLife management platform “Athena”, and in cooperation with the Information Technology Department.

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