ECDP2014 - programme & abstracts updated July 3rd 2014

12th European Congress on Digital Pathology

previously "European Congress on Telepathology & International Congress on Virtual Microscopy"

under the presidency of Catherine Guettier, honorary president Etienne Martin

@ Collège des Bernardins, Paris, France, 18-21 June 2014,

www.digitalpathology2014.org #ecdpm14

hosted by SFP - French Society of Pathology

with the contribution of ADICAP (Association for Developing Informatics in Cytology and Anatomic Pathology), F. Capron and, GFHC (French Group for Cellular Haematology), X. Troussard

Organizing Committee

Jacques Klossa and Philippe Bertheau

with Philippe Belhomme, Catherine Bor, Myriam Oger and Daniel Racoceanu, Thomas Schrader for PechaKucha sessions

and with the participation of David Ameisen, Philippe Belhomme, Philippe Camparo, Odile Crepin, Christel Daniel, Bettina Fabiani, Vincent Leymarie, Daniel Lusina, Michel Manfait, Etienne Martin, Thomas Schrader, Béatrice Vergier

Scientific Committee Chairs

Klaus Kayser, Catherine Bor, Daniel Racoceanu


Publishing

Selected articles from the congress will be published on Diagnostic Pathology special issue, www.diagnosticpathology.org, editors Catherine Bor and Myriam Oger, and on Computerized Medical Imaging and Graphics special issue, http://www.journals.elsevier.com/computerized-medical-imaging-and-graphics, editors Daniel Racoceanu and Philippe Belhomme

Feel free to read the informal abstracts proceedings as made available during the congress at: http://www.digitalpathology2014.org/_uk/proceedings.asp

Organizing Secretariat

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e-mail digitalpathology2014@theoffice.it

in collaboration with Meeting di Sara Zanazzi
From Telepathology and Digital Pathology toward Integrated Pathology

Following the long and successful biennial Congresses\(^1\) on Telepathology and Virtual Microscopy, recently organised in Venice in 2012, welcome to the 12\(^{th}\) European Congress on Digital Pathology 2014 (ECDP2014). These congresses have gradually become a reference involving a visionary college of pathologists, a dynamic scientific community, as well as a dedicated industrial consortium, specialized in digital pathology.

The ongoing transformation from **Telepathology** and **Whole Slide Imaging** to a more general **Digital Pathology** is growing slowly due to the long heritage of knowledge developed through the very powerful tools constituted by the pathologist brain aided by the microscope. However Digital Pathology is currently unconditionally used for telediagnosis, teleconsultation, e-learning, long term storage, up to image analysis, and it begins to provide valuable assistance on automated quantification particularly in the field of immunohistochemistry. At the same time, all of us, including academic and industry participants, are seriously working (as discussed during the DICOM wg26 and IHE Pathology hosted sessions) on closing the gap with **Medical Imaging** (radiology) that was quicker than microscopy to take profit from digital implementation in the business process, in the hospital integration and in using new technologies for morphological and functional observations.

To illustrate this rapid evolution in the field of Diagnostic Microscopy, the ECDP2014 congress received more than 120 communications and attendees coming from 29 countries\(^2\). Many are original communications on **Knowledge Formalization** and on **Pathology Business** integration as well on **Technology Advances**. They which will be more specifically illustrated through 3 round tables on **Telepathology Development**, **Pathology 2020 Vision** and finally on **Integrative Pathology** from Clinics and Imaging to Molecular Markers thanks to Models and innovative Molecular Biophotonics technologies that matured so as to be efficient not only *in vitro* but also *in vivo* at the Microscopy Scale.

Thank you for sharing your vision among this dynamic audience in Europe

ECDP2014 Organizing Committee

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1. Heidelberg (Germany, 1992), Paris (France, 1994), Zagreb (Croatia, 1996), Udine (Italy, 1998), Aurich (Germany, 2000), Heraklion (Greece, 2002), Poznan (Poland, 2004), Budapest (Hungary, 2006), Toledo (Spain, 2008), Vilnius (Lithuania, 2010), Venice (Italy, 2012)

2. Belgium, Brazil, Canada, China, Croatia, Czech Republic, Denmark, Egypt , Finland, France, Germany, Hungary, India, Israel, Italy, Japan, Lithuania, Mexico, the Netherlands, Norway, Poland, Qatar, Romania, Singapore, Spain, Sweden, Switzerland, United Kingdom, United states of America
with resources from MICO French ANR TECSAN project and with the kind support of:
12th European Congress on Digital Pathology, Paris, France; 18-21 June 2014

ECDP2014 Programme

Wednesday 18 June

salle 100, level 1  
salle Lexington level 1

9:00-17:00  IHE Pathology, wg 26 DICOM

CONGRESS OPENING
Chair: Frédérique Capron

17:30-19:00

SFP welcomes technology advances (Catherine Guettier) - European congresses history and progresses (Klaus Kayser and Etienne Martin) - Through the looking-glass (Jean-Claude Ameisen)

Nef level 0

19:00-21:00  WELCOME COCKTAIL

Thursday 19 June

grand auditorium, level 2  
petit auditorium, level 2

9:00-9:10  CONGRESS INTRODUCTION
Jacques Klossa, Philippe Bertheau

9:10-10:30  DIGITAL PATHOLOGY 2020
F. Capron, P. Hufnagl, M.G. Rojo
Chairs: T. Schrader, D. Racoceanu, Paul Dumas

10:30-11:00  Coffee break

11:00-12:30  A1: TELEPATHOLOGY-1
Chair: Philippe Bertheau

12:30-13:00  POSTERS PRESENTATIONS: TELEPATHOLOGY, IMAGE ANALYSIS, IT IN PATHOLOGY
Chair: Myriam Oger

13:00-14:30  Lunch

14:00-14:30  B1: IMAGE ANALYSIS-1
Chair: Daniel Racoceanu
Quantifying histopathological features and novel phenes through image analysis to stratify colorectal cancer patients (P. Caie & al.) - Nucleoli Detection using the Cascade Detector (M. Singh & al.) - Localization of Luminal Epithelium Edge in Digital Histopathology Images of IHC Stained Slides of Endometrial Biopsies (G. Li & al.) - Single Cell Segmentation with Watersheds on Highly Multiplexed Images (P. Schöffler & al.)

During round tables, myQaa application gives you the opportunity to ask questions and to vote for questions. http://ecdp14.myqaa.com
Event code: ecdn14
### Thursday 19 June

#### grand auditorium, level 2

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<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>14:30-16:05</td>
<td>A2: DIGITAL PATHOLOGY / VIRTUAL MICROSCOPY-1</td>
<td>Mircea Serbanescu</td>
<td>Automated image analysis in the study of lymphocyte subpopulation in eosinophilic oesophagitis (M. G. Rojo &amp; al.) - Visual assessment of immunohistochemical virtual slides, advantages and constraints (G. Kayser &amp; al.) - A new approach to build a composite virtual slide from multiple virtual slides of large tumor slices (M. Oger &amp; al.) - The Thatcher effect does not occur when observing rotated Whole Slide Images (WSIs &amp; al.) (J. Szymas &amp; al.) - Design and evaluation of a novel digital pathology workstation for clinical use (D. Treanor &amp; al.) - How stereology tools could improve immunochemical biomarkers assessment: example of Ki67 (C. Bor &amp; al.)</td>
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<tr>
<td>16.05-18:15</td>
<td>Coffee break</td>
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<tr>
<td>16:45-18:15</td>
<td>A3: TELEPATHOLOGY-2 / VIRTUAL MICROSCOPY-2</td>
<td>Arvidas Laurinavicius</td>
<td>The Virtual International Pathology Institute (VIPI &amp; al.) – An International Pathology Institute based upon Telepathology (K. Kayser &amp; al.) - MiViP@GE: the Eastern France digital pathology portal (J.B. Aupet &amp; al.) - Diagnostic Challenges and Advantages of International Telepathology between Two Medical Institutions (D. Borys &amp; al.) - Web-Based Oil-Immersion Whole Slide Imaging and Telediagnostics in Hematologic Treatment Planning Conference (M. Salama &amp; al.) - Digitalized whole slide imaging of oligodendrogial tumour tissue foci for diagnostic molecular 1p and 19q DNA tests (A. Eccher &amp; al.) - Whole-Slide Imaging in the Routine Diagnosis in Gynecological Pathology (J. Ordi &amp; al.)</td>
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<td>18:15-18:45</td>
<td>PECHAKUCHA SESSION</td>
<td>Thomas Schrader</td>
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#### petit auditorium, level 2

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<tr>
<td>14:30-16:05</td>
<td>B2: IT IN PATHOLOGY</td>
<td>David Ameisen</td>
<td>Preliminary results from a crowdsourcing experiment in immunohistochemistry (V. Della Mea &amp; al.) - A model based interface terminology for generic observations in Anatomic Pathology Structured Reports (G. Haroske &amp; al.) - Crowdsourcing Mitosis count: an experiment on the MITOS dataset (V. Della Mea &amp; al.) - Field testing of structured reporting using a PACS-based patient data management system (S. Seiwert &amp; al.) - Virtual Microscopy in Modern Tissue-Biobanks, the ZeBanC example (P. Hufnagl &amp; al.)</td>
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<td>18:15-18:45</td>
<td>PECHAKUCHA SESSION</td>
<td>Thomas Schrader</td>
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<td>09:00-10:30</td>
<td>FACILITATING FACTORS AND OBSTACLES FOR THE DEVELOPMENT OF TELEPATHOLOGY</td>
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<td>C. Guettier, S. Hartel, V. Leymarie, B. Têtu, C. Zhou</td>
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<td>Chairs: P. Bertheau, X. Troussard</td>
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<td>10:30-11:00</td>
<td>Coffee break</td>
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<td>11:00-12:30</td>
<td>A4: E-LEARNING                  page: 24</td>
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<td>Chair: Bernard Têtu</td>
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<td>An international university network for online teaching of pathology in French-speaking countries (P. Bertheau &amp; al.) - Creation and completion of an interactive teaching webconference in pathology (A. de la Fouchardière &amp; al.) - Discovering students' viewing behavior during a practical exam in oral pathology using software-based view path tracking for whole slide images (S. Walkowski &amp; al.) - Case exchanges and continued training formation in hematological cytology: ten years experience of an internet forum workshop (D. Lusina &amp; al.) - Online Teaching of Inflammatory Skin Pathology by a French-speaking International University Network (E. Perron &amp; al.)</td>
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<td>12:30-13:00</td>
<td>POSTERS PRESENTATIONS: TECHNOLOGY ADVANCES, E-LEARNING, QUALITY, COMPUTER AIDED DIAGNOSIS page: 78</td>
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<td>Chair: David Ameisen</td>
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<td>13:00-14:30</td>
<td>Lunch</td>
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<td>14:00-14:30</td>
<td>INDUSTRY SYMPOSIUM CLOUD PATHOLOGY page: 91</td>
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<td></td>
<td>A New Massive CPG Test: Managing a Digital Pathology Start Up</td>
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## Friday 20 June

### grand auditorium, level 2

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<tr>
<td>14:30-15:50</td>
<td><strong>A5: TECHNOLOGY ADVANCES-1</strong> page: 28</td>
<td><strong>Chair: Janusz Szymas</strong>&lt;br&gt;Confocal Fluorescent Whole Slide Imaging (V.S. Varga &amp; al.) - Incorporation of neighbourhood constraints to Fuzzy C-Means algorithm to improve the spectral histology of human tissue sections by Raman microimaging (C. Gobinet &amp; al.) - Prostate cancer assessment using stain-free, three dimensional, quantitative imaging (Genesis™200), a comparison to standard histology on whole-mount sections (C. Schwentner &amp; al.) - Digital pathology with the Fourier ptychographic microscope (R. Horstmeyer &amp; al.) - PatternQuant supported Image Analysis for IHC quantification (T. Micsik &amp; al.)</td>
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### petit auditorium, level 2

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<tr>
<td>14:30-15:50</td>
<td><strong>B5: QUALITY - 2: QUALITY CONTROL-1</strong> page: 56</td>
<td><strong>Chair: Frédérique Capron</strong>&lt;br&gt;Digital immunohistochemistry wizard: image analysis-assisted stereology tool to produce reference data set for calibration and quality control (A. Laurinavicius &amp; al.) - Accuracy of an automated vessel counting algorithm in four different tumor types (K. Marien &amp; al.) - Computer-aided HER2/neu evaluation in external quality control (eqa) of breast cancer screening programme (R. Colombari &amp; al.) - Comparative Study between Quantitative Digital Image Analysis and Fluorescence In-Situ Hybridization (FISH) of Breast Cancer Equivocal HER2 Score 2+ cases (E. Ayad &amp; al.) - FISHQuant quantification algorithm validation in the clinical molecular diagnostics (A. Csizmadia &amp; al.)</td>
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### 15:50-16:30

**Coffee break**

### 16:30-18:00

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<td>16:30-18:00</td>
<td><strong>A6: QUALITY-3: STANDARDIZATION</strong> page: 31</td>
<td><strong>Chair: Catherine Guettier</strong>&lt;br&gt;Standards and Recommendations for Digital Pathology: Image Selection and Annotation (F. Capron &amp; al.) - Standardization is needed to collaborate using whole slide images (A. Huisman &amp; al.) - Convergence in Digital Pathology data sharing: A standard recommendation for digital pathology information web-interface (Y. Sucot &amp; al.) - Digital immunohistochemistry platform for the staining variation monitoring based on integration of image and statistical analyses with laboratory information system (A. Laurinaviciene &amp; al.) - An algorithm for reducing stain variability in scanned histological slides (B. Ehteshami Bejnordi &amp; al.) - Assessment of chromosome instability predicts progressive potential of oral premalignancies (I. Otte-Holler &amp; al.)</td>
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<td>16:30-18:00</td>
<td><strong>B6: COMPUTER AIDED DIAGNOSIS SYSTEM</strong> page: 60</td>
<td><strong>Chair: Hwee Kuan Lee</strong>&lt;br&gt;Frequential versus Spatial Colour Textons for Breast TMA Classification (G. Bueno &amp; al.) - Computer-aided diagnosis from weak supervision: A benchmarking study (M. Kandemir &amp; al.) - Automated identification of cell nuclei in tissue sections (N. Timofeeva &amp; al.) - Application of Computerized Digital Image Cytometry of DNA Aneuploidy for Cervical Cancer Screening in China (C. Zhou &amp; al.) - Introduction of a cancer tissue detection method via homology (K. Nakane &amp; al.)</td>
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### 18:15-18:45

**PECHAKUCHA SESSION** page: 90

**Chair: Thomas Schrader**

### 20.00-23:00

**SOCIAL DINNER**<br>"La Maison de l’Amérique latine", 217 bd Saint Germain, Paris 7ème
Saturday 21 June

**salle LEXINGTON, level -1**

9:00-10:40

**A7: MODELS, DATA MINING AND KNOWLEDGE FORMALIZATION IN PATHOLOGY**

*Chair: Vincenzo Della Mea*

- Fractals, structural entropy and image content analysis in histopathology (K. Kayser & al.)
- Towards Ontology-Driven High-Content Image Analysis. An Operational Instantiation for Mitosis Detection for Digital Histopathology (D. Racoceanu & al.)
- Fourier parameters and fractal features distinguish between keloids and hypertrophic scars (K. Metze & al.)
- Heterogeneity assessment of histological tissue sections in whole slide images (S. Toralba & al.)
- Cell Words: A Novel Paradigm for Modeling Cells in Histopathological Images (S. Korsuk & al.)

10:40-11:10

**Coffee break**

11:10-12:30

**THE ULTIMATE GOAL: TOWARDS INTEGRATIVE PATHOLOGY**

*F. Klauschen, N. Stone; O. Piot*

*Chairs: C. Bor, V. Della Mea*

During round tables, myQaa application gives you the opportunity to ask questions and to vote for questions.

http://ecdp14.myqaa.com

Event code: ecdp14

12:30-13:00

**CONCLUDING REMARKS**

*J. Klossa, P. Bertheau*
Thursday 19 June posters session

**TELEPATHOLOGY, VIRTUAL MICROSCOPY, IMAGE ANALYSIS, IT IN PATHOLOGY**

*Chair: Myriam Oger*

- From Microscopy, Imaging to Clinical Research: A Latin American Perspective (S. Härtel & al.)
- Ki67/KL1 immunohistochemical double stains increase accuracy of Ki67 indices in breast cancer and simplify automated image analysis (P. Switten Nielsen & al.)
- Digital pathology: a new tool in Pathology department (L. Martin & al.)
- Multimodal biomarker study by PET and digital microscopy of the response to sunitinib on a luminal B-type mammary carcinoma model (B. These & al.)
- Acceptance of digital tumor board presentations in two medical institutions (D. Bors & al.)
- Virtual slides versus binocular microscope use. An orthoptic evaluation of visual strain (M.L. Ranty & al.)
- FLEXMIM: Towards efficient/effective collaborative digital pathology (P. Bertheau & al.)
- The TASTE* (Telepathological Assessment of Histopathological and Cytological Techniques) Project: Aiming to define European pathology slide technical standards (T. Tot & al.)
- Automated image analysis is superior to manual reading of HER2 expression in breast cancer (H. H. Rossing & al.)
- A segmentation method for images with subjective contours applied to immunohistochemistry-stained cell membranes (R. Pezoa & al.)
- Comparison study between TIFF and downsampled images. Automated evaluation of cytokeratin-19 immunostained scanned breast cancer tissue microarray (C. López & al.)
- Telepathology network in Ile de France: a 18-month experiment project for frozen sections (telediagnosis) and second opinions (teleexpertise) (C. Guettier & al.)
- Image analysis in virtual slides: Comparison between the expression of hormonal receptors and DNA ploidy (static cytomtry) in breast carcinoma (D. Soria-Céspedes & al.)
- A Semantic Interoperability Framework for Facilitating Telepathology (L. Traore & al.)
- Analyzing huge pathology images with open source software with an application to gliomas (C. Deroulers & al.)
- Integration tools of the digital pathology system into the research biobank management solution (L. Svanadze & al.)

Friday 20 June posters session

**TECHNOLOGY ADVANCES, E-LEARNING, QUALITY, COMPUTER AIDED DIAGNOSIS**

*Chair: David Ameisen*

- Infrared spectral imaging to automatic assessment of tumor response (H. D'inca & al.)
- 3D quantitative histopathology in the mouse brain: from mesoscopic to microscopic scale (M. Vandenberghe & al.)
- HER2 immunohistochemical assessment of breast carcinoma by image analysis in five vs. ten fields of view and its correlation with fluorescence in situ hybridization (D. Soria-Céspedes & al.)
- Typing less common ovarian tumors: A training tool based on a pattern-based algorithm applied to a set of 20 virtual slides (M. Fiche & al.)
- Virtual Slides for Teaching Pathology & Hematology (P. Flodr & al.)
- Computer-assisted diagnosis of malaria infection with an automated microscopy system (A. Grynak & al.)
- Full-field optical coherence tomography (FF OCT) of breast tissue: a new diagnostic tool for evaluation of breast proliferations? (A.T. Nadan & al.)
- Evaluation of classical and virtual slide teaching methods of practical histology (D. Krajci & al.)
- Discrepancies between diagnoses of real and virtual microscopy compared with intra and inter-observer's variation (I. Mori & al.)
- Teaching Anatomical Pathology at the University of Barcelona: Transition to Virtual Slides and Virtual Microscopes (J. Ordi & al.)
- Completely automated integrated system for prostate cancer grading (L. Molinaro & al.)
- Using a rich internet application to teach histology (R. Marée & al.)
- Virtual slides for continuing medical education in pathology (C. Guettier & al.)
- Potential of vibrational imaging in nephrology: detection of HydroxyEthyl Starch (HES) in renal tubules by Raman microspectroscopy in third generation HES associated osmotic nephrosis lesions (M. Fere & al.)
- Automatic spectral histology of human colon tissues by infrared microimaging and cluster validity indices (T. N. Q. Nguyen & al.)
- The Influence of the Microscope Lamp Filament Colour Temperature on the Process of Digital Images of Histological Slides Acquisition Standardization (T. Markiewicz & al.)
- Parallel computing in image analysis using BrainVISA software: application to histopathological staining segmentation in whole slide images (T. Delzescaux & al.)
- Digistain: A Digital Staining Instrument for Histopathology (H. Amrania & al.)
- Diagnostic question & answer thanks to Molecular Signature Detection with Multi-Modal Microscopy Scanner - M3S EU project (J. Klossa & al.)
Thursday June 19th: Digital Pathology 2020
Frédérique Capron, Paul Dumas, Peter Hufnagl, Marcial García Rojo, chaired by Thomas Schrader and Daniel Racoceanu
FRANCE, GERMANY, SINGAPORE, SPAIN

The speakers will draft a vision about the future of digital pathology, about the consequences of big data efforts, improved automatical image processing and new processes in pathology. Is it possible to establish new business models in a collaborative environment? How will the availability of genetic, social and clinical data change the diagnostic process? What are the requirements and expected next steps of development until 2020 and beyond?

The topics will be developed around: i) security, quality, annotations: tracking systems are being implemented to monitor all objects (slides, blocks, containers, forms) in a pathology department. However, images are still being managed in a rather insecure way. How do we verify that gross images correspond to the correct patient/case?, ii) what do we need to do to foster standards implementation to struggle against scarce implementation of DICOM Suupt 122 and 145, SNOMED CT, IHE Pathology technical framework, iii) communication compatibility, evolution, iv) from morphology towards pattern library / visual vocabulary consolidation and pathology databases, v) dynamic generation of medical reports associated to images, vi) considering the clinical context, vii) mega-databases (including WSI, TMA, clinical context, molecular data ...), viii) cost of moving towards digital pathology: avoiding to concentrate most resources on (radiologic) "imaging", ix) challenges of ISO xxx for anatomopathology in this context

Friday June 20th: Facilitating factors and obstacles for the development of Telepathology
Catherine Guettier, Steffen Härtel, Vincent Leymarie, Bernard Têtu, Chen Zhou, chaired by Philippe Bertheau and Xavier Troussard
CANADA, CHILE, CHINA, FRANCE, GERMANY

Each speaker will first briefly present his experience of a telepathology network in his country and in his specific medical field. The speakers will then debate together and with the audience about the main challenges that are encountered by networks coordinators and networks actors. In the context of distant 2nd opinion/expertise, frozen section diagnosis, or daily diagnosis, the potential topics that will be discussed include legal aspects, expert profile, criteria of acceptance, social and cultural aspects, economic models, technical aspects, security issues, big data and High Performance Computing. In addition, we would discuss: collaboration among government, academics and corporations, economic model and barriers for successful development of telepathology in China and other developing countries?

Saturday June 21th: The ultimate goal: towards integrative pathology
Frederik Klauschen, Olivier Piot, Nicolas Stone, chaired by Catherine Bor and Vincenzo Della Mea
FRANCE, GERMANY, ITALY, UNITED KINGDOM

This round table aims at presenting the importance of the integration approach for solving the complex problem of predictive pathology

Previously to discussion with the audience, Frederik Klauschen from La Charité will present current studies on genetic similarities observed across different cancer types and the impact on a more functional level by integrating genomic and proteomic data, then Pr Niels Grabe from Bioquant/TIGA will illustrate systems Biology application to epithelial tissue diseases interpretation and Pr Nick Stones from University of Exeter will illustrate how system biology understanding could help in exploring biological specimen for vibrational spectral data acquisition and implementing powerful automatic classifiers for early objective diagnosis.
Frozen sections diagnosis by telepathology: a pilot experiment in a two-site academic department of Pathology
C. Guettier, E. Poullier, M.-J. Redon, C. Mussini, C. Adam, E. Adnet
FRANCE

Introduction: Intraoperative diagnosis by frozen sections is used to guide the surgeon during oncological surgery. The ongoing restructuration of Pathology structures in France and the shortage of pathologists make essential an alternative solution to the presence of a pathologist on every surgical site. Telediagnosis for frozen sections has been set up since 2013 July 1st in "Hôpitaux Universitaires Paris Sud" between Paul Brousse and Bicêtre Hospitals. Both sites are distant of 2.5km and share the same Pathology lab located in Bicêtre. The goal of this pilot experiment is to evaluate the response time and diagnostic accuracy of this procedure.

Methods: From 2013/01/01 to 2013/30/06, 110 frozen section examinations for hepato-biliary and pancreatic surgery were carried out on the site Paul Brousse by a pathologist and a technician present on site. From 2013/07/11 to 2013/12/31, 139 frozen section examinations were carried out for the site Paul Brousse through the technology of virtual slides with a technician present on site and a pathologist present on the site of Bicêtre. The pathologist can assist the technician by videomacroscopy (TRIBVN) for sampling. The technician performs cutting, staining and numerization of frozen sections at X20 objective (Aperio Scanner and TRIBVN software). Virtual slides are transmitted through an intranet network to a server and are analyzed on a telepathology workstation in Bicêtre.

Results: The 139 frozen sections diagnosed by telepathology were performed during 67 surgical procedures. 26 among them required the use of videomacroscopy. A technical scanner or software failure occurred for 21 intraoperative samples during 6 surgical procedures. The problem was solved through hotline assistance in 3 cases. In the 3 other cases, the pathologist moved from Bicêtre to Paul Brousse. The mean turnaround time of a traditional frozen section (time from the arrival of the sample in the lab to the communication of the result) was 24 mn. The mean turnaround time of a telediagnosed frozen section was 41 mn (median39). Virtual slides were available to the pathologist within a mean time of 32mn. The percentage of examinations under 30mn is 100% by the traditional way and 25% by telepathology. A diagnosis discordance (benign/malignant) between frozen sections on virtual slides and paraffin sections was observed for 3 intraoperative samples.

Conclusion: This experiment demonstrates the feasibility of telepathology for frozen sections. An improvement of response time is expected through the ongoing training of all users and a constant interaction with the industrial suppliers.
expert opinion in 59.8% of cases but was in disagreement with expert opinion in 24.2% of cases. 16.0% of cases were not provided with preliminary diagnosis. The distribution of pathology cases by system or organ were: digestive system, 17.3%; gynecologic system, 16.7%; head and neck, 15.7%; bone and soft tissue, 10.4%; lung and mediastinum, 8.6%; breast, 7.6%; urinary system, 7.5%; hematopathology, 6.4%; skin, 5.2%; neuropathology, 2.5% and cytopathology, 1.3%. As for quality control, expert consultants provided assessment of quality of slide preparation and staining, and guidance for pathology quality control. Expert consultants also gave more than 40 internet-based pathology lectures through the platform.

Conclusion: our results of two years’ implementation indicated that telepathology could solve the problem of uneven distribution of pathology resources and provide a solution for countrywide pathology quality control in China. Telepathology could play an important role in improving pathology diagnosis in China.

The Eastern Québec Telepathology Network: a three year experience of clinical diagnostic services.

Bernard Têtu, Emilie Perron, Said Louahlia, Guy Paré, Marie-Claude Trudel, Julien Meyer

Introduction: The Eastern Quebec Telepathology Network (Réseau de Télépathologie de l’Est du Québec) was created to provide uniform diagnostic telepathology services in a territory of 408,760 km² with 1.7 million inhabitants. This is a report of our first 3 year experience.

Methods: The network was funded equally by the Québec ministry of Health and Canada Health Infoway, a federal telehealth funding agency. The coverage includes intraoperative consultations (IOC), expert opinions, urgent analyses and immunohistochemical (ICH) staining. The deployment of the equipment and software started in 2010 and clinical activities began in January 2011. This network comprises 24 hospitals providing oncologic surgery, of which 7 have no pathology laboratory and 4 have a pathology laboratory but no pathologist. The real-time gross evaluation during IOC was performed using a macroscopy station. The selection of the sample for IOC was performed by either a technician, a pathology assistant or the surgeon at the distant site with the on-site pathologist supervising and sketching the section needed on the screen via a drawing tablet. Slides were scanned to obtain whole-slide images.

Results: As per October 2013, 6,892 slides had been scanned for primary diagnosis / urgent biopsies; 1,131 for IOC and 1,793 for expert opinions. A recent quality assurance study showed a 98% concordance rate of IOC cases compared to the final diagnosis on paraffin material. The average turnaround time of IOC was 20 minutes. Expert opinion reports were signed out within 24 hours in 68% of cases and within 72 hours in 85%. Furthermore, a recent multi-method evaluation study of the Network demonstrated that, thanks to telepathology: 1. interruption of frozen section service was clearly prevented in hospitals with no pathologist on site; 2. two-stage surgeries and patients transfers were prevented according to surgeons and pathologists; 3. retention and recruitment of surgeons in remote hospitals were facilitated; and 4. professional isolation and insecurity among pathologists working alone was reduced. This study also demonstrated that wider adoption of telepathology for clinical use requires improvement of current technologies and that the sustainability of such a network requires better coordination and the development of a supra-regional pathology organisation. Discussion: We conclude that the Eastern Quebec Telepathology Network allowed the maintenance of rapid and high quality pathology services in a network of 24 sites disseminated on a huge territory. A second phase is underway to expand the service to other regions across the province.

ANDRAL: Open Access Solution of Tele-Expertise in Cytology

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Introduction: In hematology, the microscopic analysis of cellular morphology of blood, bone marrow, lymph node… samples remains the first important stage for diagnosis and patient follow-up. In our field, the application of information technology has allowed remarkable developments: iconographic databases, e-learning systems, automated systems of classification of blood cells,
and cytological web-review of clinical protocols. However, the cytological diagnosis itself has yet to really benefit from the great possibilities offered by modern technology. In this aim, the French speaking Group of Cellular Hematology in partnership with the College of Hematology established the open access solution of “tele-expertise” in cytology: the ANDRAL network. ANDRAL proposes remote expert decision-making support in cytology: for any transmitted request for classification an image file with a clinical/biological form is submitted. The network then allows remote classification and diagnosis to be obtained by opinion of two expert reviewers within 24h.

Materials and Methods: The ANDRAL network is accessible on www.gfhc-reseau-andral.fr. It is accessible to both hospital and the private biologists. ANDRAL is linked to a group of 45 international expert reviewers, who assure by paired review, continuous cytological care service. Globally, the system satisfies the strictest requirements regarding medical data exchange in terms of security, confidentiality, and sample/patient traceability.

Results: From October 2012 through October 2013, the network recorded more than 250 registrations with approximately 40% from private biologists, 35% from biologists practicing in a local hospital and 25% in a university hospital. 15% of the subscribers (n = 42) practiced outside of France. Over the same period, more than 120 cases were submitted: 40% were from university laboratories; 70% were de novo diagnoses; 14% were urgent requests. Image files were selections of images, 30 on average, or wide field images. The requests were variable ranging from benign to malignant pathologies and common occurrences to rare hemopathies. The average time of request management was 1h50min, and the time to obtain a classification was on average 5h30min and less than 4h for urgent requests. The ANDRAL network also features specific evaluation tools which allow for follow up activity and also allow the measure of the quality of service provided.

Discussion: Today, the assessments and the perspectives offered by ANDRAL network are real and very encouraging. In term of assessments, the constant and rapid increase of membership, the incredible motivation of all the expert reviewers and the high quality of exchanges are highlights. Several developments are further committed: collaboration with other networks and other countries (Canada, ...) and the development of a permanent economically feasible medical model.

Conclusion: For the hematologists involved in morphological diagnoses, the ANDRAL network is a working example of cooperative function of which the first results are very encouraging.

Is smartphone an accurate tool for telepathology? A prospective study on second opinions in pathology
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FRANCE

Introduction: Pathologists are few in number. However, their role is increasingly enhanced in modern medicine. A recent report has shown that there are fewer and older French pathologists than some years ago, but the next generation of young pathologists is ready. Therefore, in the near future there will be young, inexperienced and isolated pathologists.

Second opinions in pathology are frequent, accounting for about 200,000 cases per year, but causing delay in patient care. With the rise of telemedicine, digital pathology is developing, helping pathologists to reduce this delay. Many technologies have been developed (i.e. scanners, digital microscopes and digital slide reading consoles) but their use is limited by their size and cost.

The aim of this study was to determine if small, cheap and easy-to-use devices such as the smartphone could help pathologists in their daily practice.

Methods: Three different kinds of second opinion were assessed in this prospective study: 1) Frozen sections; 2) "in house consultations", and 3) external opinions. Interest or troublemaking areas were selected by traditional optical microscope. Digital images were taken with iPhone 5 (Apple, Cupertino, CA, USA) with the help of a Skylight device adapter (Skylightscope, Oakland, CA, USA) and then transferred to a MacBook Pro notebook (Apple, Cupertino, CA, USA). Pictures were first shown to an experienced pathologist giving clinical context; Pathologists were able to discuss while looking at the pictures, as they would do by phone. Opinion was collected and then histological slides were read. Diagnosis performed on digital pictures was compared to whole histological slide interpretation.
**Results:** Sixty-six cases were included: 41 frozen sections, 6 in-house consultation opinions and 19 external opinions. Average number of photos was 0.32 for macroscopy, 6.55 for microscopy, 0.14 for special stains, and 1.12 for immunochemistry.

In 93.9% of cases (62/66), digital pictures lead to diagnosis, 3% (2/66) to further investigation and 3% (2/66) were insufficient to conclude. After whole slide interpretation, pathologists confirmed their diagnosis in 96.9% (64/66) of cases. They asked for further investigation in 1.5% (1/66) of cases and changed the final diagnosis in 1.5% (1/66) of cases.

**Discussion:** Our pilot study demonstrated that smartphone can be an efficient tool for pathologists. Its accuracy is near to conventional histological interpretation and, compared to other telepathology tools, is much easier, cheaper and faster to use. The most common restriction in digital pathology is choosing the selected areas: pathologists are always concerned about missing areas of interest, but the simplicity and rapidity of the smartphone far outweighs these concerns. Security and confidentiality in data storage and transmission are a pre-requisite in legal terms. Further evidence is required to back up our findings, but there are already many reasons to be excited about digital pathology transmission by email and Short Messenger Service.
Automated image analysis in the study of lymphocyte subpopulation in eosinophilic oesophagitis

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Introduction: Eosinophilic oesophagitis is characterized by the presence of eosinophils in oesophageal mucosa. Since normal mucosa does not show these cells, their presence is always pathological, and is usually associated with gastroesophageal reflux disease (GERD), oesophageal eosinophilia due to proton pump inhibitors (PPIs), and eosinophilic oesophagitis (EoE). The American College of Gastroenterologists include as minor criteria for diagnosis of eosinophilic oesophagitis the increase in the number of lymphocytes and mast cell in oesophageal mucosa.

Some previous studies did not find significant differences in lymphocytes count (FoxP3, CD8, and CD4) between EoE and GERD. The aim of this study is to compare the inflammatory pattern of (EoE) with (GERD) and normal mucosa, to assess treatment response after diet free of specific food (legume, egg, milk, etc.), and to evaluate different location biopsies (proximal, medium, distal oesophagus).

Methods: From 2010 to 2013, 195 oesophageal biopsies from 38 patients were randomly selected from pathology department information database. From these, 15 patients were diagnosed as GERD and 23 patients were diagnosed of EoE. Patients from EoE were treated with food exclusion diets and multiple biopsies (from 1 to 9 biopsies) were taken during follow up (from 6 months to 48 months).

Since conventional parameters to evaluate eosinophilic oesophagitis are prone to subject interpretation by pathologists, in order to evaluate inflammatory pattern with objective criteria, we performed inflammatory cell count using different monoclonal antibodies, to detect lymphocyte subpopulation (CD3, CD20, CD4, CD8, and FoxP3), CD1a dendritic cells, and mast cells (c-kit/CD117).

Immunohistochemical expression (DAB) of those markers was quantified with the Leica Aperio Positive Pixel Count algorithm version 9.1, as previously described (Brazdziute, 2011). Two approaches were used with this algorithm: whole slide counting and “hot spots” (selecting a 0.5 mm² area with highest cell count)

Results: Eosinophils manual count correlated much better with CD3 and CD8 count using hot spots approach than with a whole slide approach. The rest of the immunohistochemical markers did not show good correlation with Eosinophil count, independently of the used approach.

Positive Pixel Count algorithm applied to whole slide was useful to distinguish EoE from GERD. 85% EoE biopsies showed more than 2.5% of the whole tissue area (excluding stroma) stained with CD3. GERD patients showed between 0.2 and 2.23% of the whole tissue area stained with CD3. With CD4, 93% of EoE biopsies showed over 0.20% marked areas, whereas GERD patients showed from 0.05 to 0.19% CD4 positive areas. A 95% of biopsies with EoE showed CD8 positive areas over 2%. All patients with GERD showed less the 1% of marked area with CD8.

Considering CD3, CD4 and CD8 counts, Positive Pixel Count algorithm has a positive predictive value of 98% and a negative predictive value of 83% in the diagnosis of eosinophilic oesophagitis. CD20, CD1a, and c-kit (CD117) were not useful to distinguish EoE from GERD using automatic image analysis.

A simple a free tool like Leica Aperio Positive Pixel Count is a useful tool validated to quantify area and intensities of positive and negative immunohistochemistry staining. Automatic CD3, CD4 and CD8 staining quantification in whole slides is a useful tool in the diagnosis and follow up of eosinophilic oesophagitis.

Visual assessment of immunohistochemical virtual slides – advantages and constraints

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The use of virtual slides has been implemented in pathology research for more than a decade. Automation of quantification processes offers new possibilities not only for high throughput analyses but also for commercial vendors. Up to now it has only been assumed that assessment slides by conventional bright field microscopy and by virtual microscopy deliver equal results. To prove this hypothesis we have investigated a series of TMAs stained by immunohistochemical double stains for inter-methodological agreement.
In our study of 151 lymph node metastases of non-small cell lung cancer patients, we could show high agreement of the obtained results. Correlation coefficients have been calculated of > 0.9. We can therefore prove that visual assessment of virtual slides delivers results equal to conventional microscopy and that the results obtained are within the range of expected intra-observer variability.

On the other hand, virtual slides are processed images. Algorithms used to optimize scanned images include color and illumination normalization, gamma correction and image compression. Here we review the pros and cons of these image processing algorithms and their impact on visual assessment of immunohistological stained virtual slides.

**A new approach to build a composite virtual slide from multiple virtual slides of large tumor slices**

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**FRANCE**

**Context:** Currently available microscopic scanners produce whole slide images at various resolutions from histological sections. Having the entire section of the tumor and adjacent tissue at his disposal up to the 20x magnification, the pathologist can visually evaluate for instance the quality of the tumor margins, the invasion and growth of the lesion as well as the compression and alteration of adjacent structures. Nevertheless, acquisition area and so visualization of large tissue samples are limited by the standardized size of glass slides, used daily in pathology departments. The proposed solution has been developed to build composite virtual slides from images of large tumor fragments.

**Material and Methods:** Images of HES or immunostained histological sections of carefully labeled fragments from a representative slice of breast carcinoma were acquired with a digital slide scanner at a magnification of 20x. Taking into account the large size of the whole slide images, a two resolution procedure has been developed. A first composite image is built at a low resolution. The user is allowed to correct the automatic stitching of the fragments if needed. Then, the final composite virtual slide is assembled at full resolution by reference to the parameters obtained at low resolution. The final image is saved in a pyramidal BigTiff file format.

The program has been tested on several tumor slices. A correlation quality control has been done on five images. The correlation coefficient was computed between the original images and the corresponding rebuilt images, previously artificially cut into four to seventeen pieces.

Results: A simple protocol for macroscopy and histology has been defined in order to improve the final result of the composite virtual slide building. More than one hundred tumor slices from twenty eight surgical specimens, cut into two to twenty six pieces, were reconstructed. The mean size of slice was 0.5 cm² for a mean file size of 56GB. A median of 98.71% is obtained by computing the correlation coefficients between native and reconstructed images for quality control.

Conclusions: The proposed method is effective but must have further developments in order to be user friendly and to improve its speed for daily practice. Funded by the European Union, "Europe is involved in Normandy" with the European Regional development fund.

The Thatcher effect does not occur when observing rotated Whole Slide Images (WSIs)

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**Introduction:** The Thatcher effect is a phenomenon where it becomes more difficult to detect local changes in upside-down face pictures. This is thought to be due to specific psychological cognitive modules involved in face perception which are tuned to recognize especially upright faces. Pathologists diagnose diseases and classify tumors based on subtle visual cues that are difficult to determine. Some researchers compare the experience of spotting a tumor with recognizing somebody in a photo. If it is so, it raises a question whether the Thatcher illusion also occurs when one observes histological images.

**Methods:** We conduct practical examination in oral pathology using virtual slide images (WSIs). Each practical exam consists of 50 multiple-choice questions per student. Every question is displayed together with the adequate WSI and students have to provide an answer based on the interpretation of this WSI. Since 2009, we have applied a random 180 degrees rotation of 50% of WSIs to prevent the students from memorizing only small icons of WSIs during laboratory practicals. In the present study we analyze the examination results of 456 dental students.
evaluated between 2009 and 2013. During this period, the students made 22,800 histological diagnoses based on the observation of unrotated or rotated WSIs. We first provide calculations of total average correct answer rates, and the average correct answer rates for the datasets of unrotated and rotated WSIs separately. Then, we present descriptive statistics and correlations for the analyzed datasets.

**Results:** There were small differences, however statistically insignificant, between the total average correct answer rates and the average correct answer rates for students evaluating slides from either of the cohorts (not rotated or rotated WSIs). In the years 2010 and 2011, the average positive answer rates were even slightly higher for students evaluating rotated slides than for those evaluating slides from the non-rotated cohort. We did not find any single WSI giving rise to a particularly low correct answer rate after its rotation, nor any single student with an exceptionally low number of correct answers after rotation of WSIs.

**Conclusion:** Our results show that rotation of WSIs through 180 degrees does not influence the ability of the observers to correctly recognize histological features of the evaluated specimens. So far, we can speculate that the basic mechanisms of face and histological features recognition are different. Further research into the Thatcher and other illusions might help determine whether histological slide perception and recognition is a serial or a parallel process, and whether histological diagnosis is done based on recognition of tumor-specific details allowing the pathologist to distinguish some particular characteristics of the tumor or rather by some perceptual gestalt.

**Keywords:** Thatcher illusion, WSI, oral pathology

**Design and evaluation of a novel digital pathology workstation for clinical use**

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**UNITED KINGDOM**

**Introduction:** If digital pathology is to be widely adopted in clinical practice, it must be acceptable to pathologists and have proven efficiency. However current digital pathology workstations are significantly slower than the light microscope - as much as 60% slower in our previous experimental evaluations. We set out to make a digital pathology workstation (the Leeds Virtual Microscope, LVM) which is as fast as the light microscope.

**Methods:** We describe (1) formal qualitative study of pathologists work activities using observation and interviews (2) quantitative evaluation of microscopy including time and motion studies, tracking at the microscope, and quantitative evaluation of laboratory workload and (3) experimental evaluation in controlled counterbalanced trials with 12 pathologists of a novel digital pathology workstation.

**Results:** The design process and evolution over 7 years of the LVM is described from a 50 megapixel powerwall device to a desktop workstation practicable for clinical use. The final system is a 9 megapixel workstation with high performance image manipulation, pathologist-centred workflow, and a novel case-based design with on-screen metadata.

Extensive experimental evaluation showed that the LVM workstation is both acceptable to pathologists and equivalent to the light microscope in diagnostic time (median normalised time to diagnosis 96.5% on LVM and 112% on microscope). Detailed behavioural analysis of shows that pathologists spent more time looking at the images on the LVM (79% of time on LVM vs. 63% on microscope, P < 0.05), and used the LVM differently to the microscope, making almost twice as many additional slide views on the LVM (P < 0.05).

**Conclusion:** A systematic multi-disciplinary approach to workstation design was successful. The resulting novel digital pathology workstation was both acceptable to pathologists and as efficient as the light microscope.
How stereology tools could improve immunohistochemical biomarkers assessment: example of Ki67


Background: Translational research contributes to identify theranostic biomarkers more often by molecular techniques. Some of them, after correlation studies, are now detected by immunochemistry. Personalized medicine, will induce for the next years the need for a more accurate evaluation of immunohistochemical biomarkers by the pathologists in order to find the significative values and the methodology to improve their reproductibility. This study aims to evaluate the labelling index of Ki67, with a methodologic approach, using stereology tools and digital pathology.

Methods: Study was performed on a series of 92 women with the same neo-adjuvant chemotherapy for invasive breast carcinomas, positive for one or two hormonal receptor and negative for HER2. Evaluation of pathologic complete response (pCR) was made on surgical specimens. Estimation of Ki67 labelling index was performed on pretherapeutic core-biopsies using a standardized immunochemistry procedure. We compare two methods of quantification by visual count under microscope by a pathologist, eyeball and 100 to 400 cells count to a manual count on corresponding digitized slides using stereology box grids.

Results: Best correlation for mean value is obtained between stereology count on digital slides and visual Eye Ball count on microscope(r=0.87, p<0.0001). Neither visual Hotspot, nor visual count from 100 to 400 cells on microscope is correlated with pCR. Correlation is found for Ki67 stereology value and pCR (p=0,03).

Conclusion: This comparative evaluation of Ki67 labelling index was an analytic way to define different bias at each step of the visual quantification by the pathologist. This study highlights the strength of random sampling and counting using stereological rules.

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A3: Telepathology-2 & Virtual Microscopy-2

The Virtual International Pathology Institute (VIPI) – An International Pathology Institute based upon Telepathology
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Introduction: The roots of telepathology range back for 25 years. Telepathology has matured and is applied in online and offline algorithms. An international solely electronically based medical institute with patient-directed services has not been implemented to our knowledge until 2012, and still remains a blank area in both medical and financial issues. The Virtual International Pathology Institute (http://www.diagnomx.eu/vipi) which we herein explain and describe has opened a new area in telemedical diagnostic services with surprisingly new results and experiences.

Foundation and Aims: VIPI was founded by 65 experienced pathologists who are working in different internationally distributed institutes / departments of pathology in 2012. The founders signed strict bylaw regulations and agreed to view, interpret and diagnose microscopic and other images electronically in order to serve for tissue-based diagnosis. All demands in surgical pathology are covered, starting for expert consultation to liable diagnosis, judgement of laboratory and image quality to interpretation of automated immunohistochemical measurements provided by an integrated image analysing system, to train young colleagues, and to pre-review scientific case reports to be submitted to open access pathology journals. Services for glass slide immunohistochemistry combined with virtual evaluation performed by VIPI members and automated measurements including scoring are offered too.

Internal organization: VIPI has been constructed and implemented according to the experiences of members during their services for the Salomon Islands in 2002 – 2006. A separate handling of medical, formal, and potentially financial issues was implemented in a fully democratic manner as well as the medical director and advisory board who are responsible for diagnosis performance, fast response, education, and update of recent medical research. The technical director steers the technology and financial issues.

Technology and Implementation: The principle idea is network based algorithms comparable to Grid technology or cloud computing. The backbone of VIPI is a specifically tuned php based forum that itself is divided in five independent channels. These comprise acute cases, closed cases, review cases, education – atlas, and tissue submission. Its specific services are directed to external servers and include: access to the NIH library in the USA, several open access peer reviewed journals, automated image quality evaluation and control, automated language translation, automated IHC measurements, access to an image lung atlas, and outsourcing of immunohistochemistry.

Experiences, submitted material, reliability, accuracy: The routine work of VIPI started on January 1, 2013. The presented statistical analysis covers the period January 1, 2013 to December 31, 2013. A total of approximately 500 cases have been submitted for consultation, and additional 20 cases for review. The minimum period between submission and diagnosis amounted to less than 5 minutes. All responses took place within 1 – 2 days after submission. A definite diagnosis was requested in more than 90% of submission, and confirmed by 5 – 6 members per cases at average.

Cytology and biopsies cover more than 60% of cases, and about 40% are surgical specimens. IHC was demanded in approximately 10 cases. Results of automated image measurements can be performed for images acquired from HE, IHC, FISH, and AgNoR stained glass slides.

Perspectives: From the medical and scientific point of view VIPI has proven that solely electronically provided tissue-based diagnosis is equivalent to conventional surgical pathology. In addition, its service is fast, includes contemporary multiple expert consultations, assists conventional pathology institutes in periods of missing personnel, and performs automated internal quality evaluations of both, image quality and quantification.

MiViP@GE: the Eastern France digital pathology portal.
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FRANCE

Introduction: Eastern France Cancéropôle (Cancéropôle Grand-Est, CGE) is setting up a digital pathology portal, a project led by pathologists to share high definition digitized slides. This tool will allow participating members
to share in a control manner microscopic images as produced by high resolution scanners together with clinical annotations as required for research, teaching or diagnosis applications. This project thus stands out by the ambition of covering the broad possibilities brought by modern digital pathology.

**Material and Methods:** The project was initiated in November 2011 and conducted by a dedicated full time software engineer under the supervision of a medical coordinator for the University Hospital of Besançon chosen as a pilot site. The first phase consisted in the evaluation of commercially available scanning equipments and produced images sharing solutions, via on-site testing. Specifications were validated by an ad hoc working group and shared with the other participating sites: the MiViP@GE portal will bring clinicians and researchers a sharing facility for clinical and experimental data on a project basis.

**Results and discussion:** Advances in scanning and communication technologies opens an avenue for digital pathology that extends the traditional collaborative microscope to larger networks, facilitating the access to teaching collections, the independent reading of slides and the recourse to experts. Digitization offers long-term storage of high-definition images and the possibility to review multicentric collections past the recruitment phase without the need to centralize all physical slides. Associated data will be accessible according to the project based profile granted to the partner, and comprise various types of documents.

Implementation of this project targets three main fields:

1. **Research:** virtual slides are assigned to specific CGE research projects. The first project involves Besançon University Hospital and the DKFZ (Heidelberg) with shared data consisting in microscope slides and NGS DNA sequencing results.

2. **Teaching:** the portal has been tested in a dermatology course with an attendance of 50 students. Slides were made available prior to the course. Of note is the possibility to enrich the teaching collections via a connection to ongoing initiatives such as the “International university network for online teaching of pathology in french-speaking countries”.

3. **Diagnostic:** requests for second reading in support to expertise networks. This tool will notably be key in promoting an inter-regional expert center common to the University Hospitals of Besançon and Dijon, applied to lymphomas and brain tumors. Extension to whole Eastern France is considered. This application field involves higher layers of security and 2-factor authentication solutions are evaluated.

**Conclusion:** Digital pathology has emerged owing to new generation scanners and sharing software supporting virtual slides streaming over the Internet. The CGE initiative aims at taking full benefit of these new technologies via the implementation of a portal opened to Eastern-France researchers, as well as their worldwide collaborators. Particular attention is brought to the fast developments in this field, with the concern of stepping on solutions ensuring the highest compatibility level and amenable to the integration of future progress. Opportunities and issues are discussed within a nation-wide working group that gathers the digital pathology promoters within each Cancéropôle, an initiative extremely fruitful in that respect.

**Diagnostic Challenges and Advantages of International Telepathology between Two Medical Institutions**

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**UNITED STATES OF AMERICA**

**Introduction:** Digital pathology is an evolving field with immense value. Though used primarily as an educational or research tool, digital imaging is starting to be incorporated into daily pathology practice and has already been implemented in certain remote areas with limited access to pathologists. The aim of this study is to demonstrate the diagnostic accuracy of telepathology used in the setting of an actual surgical pathology consultation between two medical centers from different countries and time zones.

**Design:** Over a twenty-five day period, slides for bone and soft tissue subspecialty cases were captured with the Aperio ScanScope® CS whole slide scanner at University of California Davis (UCD) upon receipt from the histology department. The slides for each case were viewed virtually by a pathologist with expertise in bone and soft tissue located at Rizzoli Orthopedic Institute, Bologna, Italy using the Aperio Spectrum WebScope. The pathologist had secure access to the UCD Laboratory Information System (LIS), electronic medical records, and radiology images. Case discussions and requests for deeper sections and immunohistochemistry were accomplished by secure hospital email. The glass slides for these cases were later viewed by...
light microscopy in a single-blinded fashion by three pathologists to evaluate for concurrence or discrepant findings with the originally reported results.

**Results:** Fifty-two cases were scanned and evaluated virtually, providing a primary diagnosis in fifty-one cases and a second opinion in one case. The mean time between scanning cases and reporting results was 2.29 days. The majority of cases (69.2%) were evaluated and reported within one day, either on the day they were scanned (8/52) or by the following day (28/52). One histologic discrepancy (1.9%) was identified upon light microscopic review. The virtual image for the discrepant case was reexamined, and the image was found to be of poor quality.

**Conclusion:** Our international telepathology experience has shown that digital pathology is adequate for primary diagnosis and consultation and can be included in daily pathology practice without delaying diagnosis. However, image quality should be closely monitored to ensure accurate diagnosis. This study also shows that digital pathology can bridge the temporal and geographic gaps between medical centers from different countries and time zones in an accurate and timely fashion, providing access to expert subspecialists that would otherwise not be within reach.

**Web-Based Oil-Immersion Whole Slide Imaging and Telediagnostics in Hematologic Treatment Planning Conference**

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**UNITED STATES OF AMERICA**

**Introduction:** Whole slide imaging (WSI) is a key innovative technology that enables telemedicine platform and allows slides (tissue sections, bone marrow aspirates, blood smears) to be scanned at high resolution (oil immersion) for presentation at the teleconference. To the best of our knowledge, there is no peer reviewed studies objectively measuring the perceived increased satisfaction or the perceived increased or decreased time required to scan slides and annotate fields of interest for presentation at tumor board (TB). Here in, we present our experience with introduction of oil immersion WSI for blood/marrow aspirate smears in hematology TB /clinical care conferences and compare its performance to a microscope projection system for time efficiency and clinician satisfaction.

**Material and Methods:** Whole-Using Aperio XT & CS-O slide scanners, lymph nodes and core biopsies were scanned at 20X magnification and blood/marrow smears at 100X under oil immersion and uploaded to an online library (Aperio eSlide Manager) with areas of interest annotated digitally to be displayed via a standard web browser. Time required to identify scanning areas and annotate as well as time for presentation was compared to microscope projection (MP), which required moving to cells of interest on each slide on a microscope connected to a video camera/projector. A 10-point evaluation survey was used to assess clinical staff satisfaction with each presentation method.

**Results and Discussion:** There was a reduction of the average presentation time from 1.3 min to 0.5 min per slide in MP and WSI, respectively. In addition, pathologist’s pre-conference preparation time using WSI was reduced by 20% in average when compared to total time with MP. Technician time for oil immersion slide scanning averaged 9.7 min/slide (range 5-23 min depending on size of scan area). Survey results (78% response rate) showed a significant increase in satisfaction by clinical attendees with regard to image quality (p=0.001), efficiency of presentation of pertinent findings (p<0.0001), aid in clinical decision-making (p=0.008) and overall satisfaction (p=0.002) regarding pathology presentation (mean of differences 2.9-5.7 for each criteria; t-test). A majority of respondents also noted decreased motion sickness with WSI.

**Conclusion:** WSI provides higher quality images compared to traditional MP and significantly increases clinician satisfaction. WSI streamlines preparation for TB by permitting prior slide contents selection, and results in greater efficiency during TB presentation. WSI shifts time requirements to technicians and increases efficiency for pathologists and clinicians, resulting in an improved overall TB experience.
Digitalized whole slide imaging of oligodendrogial tumour tissue foci for diagnostic molecular 1p and 19q DNA tests.

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ITALY

Background: Gliomas are the most frequent tumors of the central nervous system. Molecular abnormalities are playing an increasingly prominent role in the management of the patient by defining molecular subgroups with prognostic and predictive clinical significance. Among molecular abnormalities in gliomas, the codeletion of chromosome arms 1p and 19q is associated with a better prognosis and is predictive of response to therapy. However, fluorescent in situ hybridization (FISH), which is the gold standard to detect such anomalies, is available only in few referral neuropathology center. Thus, histological diagnosis and FISH test are often performed independently in different institutions and finding appropriate tumoral areas matching to those where FISH test is performed, is often critical, potentially causing false results. We aimed to overcome the tissue mismatch.

Methods: Histologic slides from 40 cases grade II-III oligodendroglioma or oligoastrocytoma were digitalized using the D-Sight instrument and visualized on-line by two pathologists staying in two different locations using a telepathology network. Each case was jointly evaluated for the diagnosis according to WHO classification and the appropriate tumor area for FISH analysis was selected.

Results: An average of 7 slides for each case were digitalized and evaluated by two pathologists in distant institutions. In 37 of the 40 cases (92%) the choice of tumor area, selected independently by each pathologists, resulted equal with an almost perfect concordance (0.81 kappa statistic) for molecular tests. 5 cases of 37 (13%) were scarce bioptic material, entirely present in one block. In the remaining three cases (8%) the accordance was difficult due to the peculiar morphology of the tumor, characterized by questionable presence of oligodendrogial features. A live online session was then organized to discuss the cases and jointly choose the representative slides for questionable cases on all slides.

Conclusion: Inter-institution sharing of virtual histopathology slides facilitates both the diagnostic process and the appropriate selection of material for molecular analysis. This saves time and budget linked to transfer of materials between institutions as the materials are evaluated for their appropriateness beforehand any shipment is made.

Whole-Slide Imaging in the Routine Diagnosis in Gynecological Pathology

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SPAIN

Background and aim: Whole slide imaging (WSI) is the process of digitizing glass slides, which enables the examination of pathology samples on a computer screen in a manner comparable to light microscopy. Although WSI has been used for different applications its use in routine diagnosis is still limited. This study aims to determine the accuracy of interpretation using WSI in the routine diagnosis of gynecological specimens as compared with conventional light microscopy (CLM), to understand the technology limits and possible interpretative pitfalls.

Methods: All gynecological biopsies (including small biopsies and surgical specimens) received at the department of pathology of the Hospital Clinic of Barcelona, a tertiary University Hospital in July and August 2013, were analyzed blindly by two gynecological pathologists. One of them performed the diagnosis using CLM. For WSI, H&E slides were digitized in a Ventana iScan HT (Roche diagnostics) at 20x. All discrepancies were reviewed in double headed CLM and a final consensus diagnosis was established. The discrepancies were classified according to a modified Goldman classification as major (significant differences in clinical management or benign vs. malignant) or minor (no or minor clinical relevance). The results were evaluated by weighted Kappa statistics for two observations.

Results: A total of 452 cases, consisting of 1253 glass slides, were evaluated; 48.2% of the biopsies were normal or had reactive lesions, 28.6% had benign tumors, 4.2% had low-grade premalignant lesions, 10.6% had high-grade premalignant lesions and 8.4% had malignant tumors. In 94.2% of the biopsies there was a complete agreement between WSI and CLM interpretations. Major discrepancies were observed in 9 cases (2.0%).
Seven cases consisted of small lesions (five high grade squamous intraepithelial lesions [HSIL] of the cervix, one lymph node micrometastasis of an ovarian carcinoma) underdiagnosed (3 cases) or missed (4 cases) in the WSI evaluation, and two cases consisted of small HSIL lesions missed or underdiagnosed (1 each) in the CLM evaluation. Minor discrepancies accounted for 3.8% of the biopsies. Interobserver agreement for WSI and CLM evaluations was at the almost perfect level (kappa value 0.914; 95%CI: 0.879-0.939). Interobserver agreement increased during the study period: kappa value 0.904; 95%CI: 0.863-0.945 in the first month, and kappa value 0.959; 95% CI: 0.896-1.00 in the second month.

**Conclusion:** Diagnosis of gynecological specimens by WSI is accurate. Routine diagnosis and digital archiving of gynecological specimens by WSI may be confidently introduced in departments of pathology.
**A4: E- Learning**

**An international university network for online teaching of pathology in French-speaking countries**

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**CANADA, FRANCE SWITZERLAND**

**Introduction**: Building online courses is a highly time consuming task for teachers of a single university. Universities working alone create high-quality courses but often cannot cover all pathological fields. Moreover this often leads to duplication of contents among universities, representing a big waste of teacher time and energy. We initiated in 2011 a French university network for building mutualized online teaching pathology cases, and this network has been extended in 2012 to Quebec and Switzerland.

**Method**: Twenty French universities (see & for details), University Laval in Quebec and University of Lausanne in Switzerland are associated to this project. One e-learning Moodle platform (http://moodle.sorbonne-paris-cite.fr/) contains texts with URL pointing toward virtual slides that are decentralized in several universities. Each university has the responsibility of its own slide scanning, slide storage and online display with virtual slide viewers. The Moodle website is hosted by PRES Sorbonne Paris Cité, and financial supports for hardware have been obtained from UNF3S (http://www.unf3s.org/) and from PRES Sorbonne Paris Cité. Financial support for international fellowships has been obtained from CFQCU (http://www.cfqcu.org/).

**Results**: The Moodle interface has been explained to pathology teachers using web-based conferences with screen sharing. The teachers added then contents such as clinical cases, self-evaluations and other media organized in several sections by student levels and pathological fields. Contents can be used as online learning or online preparation of subsequent courses in classrooms. In autumn 2013, one resident from Quebec spent 6 weeks in France and Switzerland and created original contents in inflammatory skin pathology.

These contents are currently being validated by senior teachers and will be opened to pathology residents in spring 2014. All contents of the website can be accessed for free. Most contents just require anonymous connection but some specific fields, especially those containing pictures obtained from patients who agreed for a teaching use only, require personal identification of the students. Also, students have to register to access Moodle tests. All contents are written in French but one case has been translated into English to illustrate this communication (http://moodle.sorbonne-paris-cite.fr/mod/page/view.php?id=261) (use “login as a guest”). The Moodle test module allows many types of shared questions, making it easy to create personalized tests. Contents that are opened to students have been validated by an editorial committee composed of colleagues from the participating institutions.

**Conclusions**: Future developments include other international fellowships, the next one being scheduled for one French resident from May to October 2014 in Quebec, with a study program centered on lung and breast pathology. It must be kept in mind that these e-learning programs highly depend on teachers’ time, not only at these early steps but also later to update the contents. We believe that funding resident fellowships for developing online pathological teaching contents is a win-win situation, highly beneficial for the resident who will improve his knowledge and way of thinking, highly beneficial for the teachers who will less worry about access rights or image formats, and finally highly beneficial for the students who will get courses fully adapted to their practice.

**Creation and completion of an interactive teaching webconference in pathology**

**Arnaud de la Fouchardière**

**FRANCE**

We have elaborated an interactive pathology teaching webconference using virtual slides. The target attendees were senior pathologists in private practice. The preparation included the selection and scanning of slides. The areas of interest were chosen in each slide and the session completely scripted. The date and hour of the session were chosen ahead of time (maximum availability of target audience chosen). A free invitation was sent by mail, giving all useful details on technical
parameters of connection to the webconference. The session lasts one hour with the opportunity to connect half an hour before start. The organizer presents live and invites the audience to participate through a chatbox system that can either be seen by only the organizer or by all participants. It can be a diagnostic question: the audience gives their answers and comments are made by the organizer when all participants have answered. It can also be other direct questions and the organizer can also be questioned on some details. Cases can be completed with scanned antibodies and the teaching can also involve ancillary techniques. At the end of each case a thorough list of major teaching points is given. An open discussion follows if necessary.

The IT environment used was completely mobile: laptop computer using a wifi connection, virtual slides uploaded from a remote server, mobile phone for voice conference.

We have performed 4 sessions on an 18 month span. 109 pathologists were invited and 68 participated. Three separate cases were studied using a total of 14 slides. For most users it was their first webconference. A majority were very satisfied of this type of teaching on evaluation forms sent after the conference.

The selection of cases and the preparation beforehand are paramount to the success of this teaching type.

**Discovering students’ viewing behavior during a practical exam in oral pathology using software-based view path tracking for whole slide images**

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FINLAND, POLAND

**Introduction:** The technology of whole slide images (WSIs) used in education allows not only to improve the learning and assessment process but also to provide new insights on how histological slides are viewed by students when answering questions during a practical exam in oral pathology. To discover viewing patterns, we created software infrastructure which tracks students’ viewing behavior, used it during an exam and analyzed the data gathered.

**Material and Methods:** Our method utilized in this work does not require any specialized equipment and is scalable for large volumes of users and WSIs. Students participating in the exam in oral pathology (N=85) used functionality implemented on a virtual microscopy software platform (WebMicroscope, Helsinki, Finland) to view whole slide images (50 WSIs per student). It allows to dynamically track view paths across the whole WSI area and all zoom levels, while collecting the viewing behavior data centrally from many simultaneous WSI users. Whenever a student stopped panning and zooming for a while, a record was saved in the central database. It contained coordinates of the area viewed, timestamp, and student and question identifiers.

Gathered data was later visualized – the generated images and animations had view fields and paths marked on WSI thumbnails. These visualizations enabled analysis of the viewing behavior of single students and comparison of two or all students viewing the same WSI. Finally, statistics were designed and calculated to automatically discover certain viewing patterns for multiple students and multiple WSIs. Measures selected for the final analysis included average zoom level on which a WSI was viewed, dispersion of view fields, total viewing time, total number of view fields and a value describing how much a student was focused on diagnostic areas of a slide. In both visual and numerical analyses, view paths for correct and incorrect answers were distinguished to see a potential correlation between viewing behavior and correctness of the answers given by students.

**Results and Discussion:** The exam was conducted successfully using the new software infrastructure. On average, there were about 750 view field records stored per student in an exam session. Some characteristic viewing patterns for selected questions and students were discovered visually, based on the generated visualizations. Calculated statistics confirmed certain observations. Moreover, the numbers allowed generalizing some findings across many students or WSIs. The overall results include calculations showing that in most exam questions students answering incorrectly tended to spend more time on interpreting WSIs and go through more view fields, which were more dispersed – all compared to students with correct answers.

**Conclusion:** Proposed and implemented view path tracking appeared to be a useful method of discovering how students view WSIs during an exam in pathology. Our approach allowed us to track a significant number of students, simultaneously viewing many WSIs, and provided valuable data, which was analyzed. Visualizations and calculated statistics resulted in multiple insights on WSI viewing behavior. The possibility of discovering such knowledge is another benefit from using WSI technology in medical education.
Case exchanges and continued training formation in hematological cytology: ten years experience of an internet forum workshop
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FRANCE

Introduction: Blood, bone marrow, lymph node cytology are key stages in the diagnosis of malignant hemopathies as they are the first important stage in biological investigation and enable the diagnosis either to be made or to orientate it and consequently indicate the necessary complementary diagnostic or prognostic tests.

The cytological diagnosis procedure depends on the morphological analysis, taking into account the available clinical and biological data and the CBC (complete blood count) results. Beyond the initial training, the skills and experience of the biologist's involved, cytological comparisons contribute to increasing and testing their knowledge.

Materials and Methods: Modeled on meetings for cytological training organized by the association A.R.C.H.E. (Research group for cytological hematology and teaching) which was initiated by Prof. Flandrin and considering the logistical difficulties that a large scale exchange of slides represented, a forum workshop “Questions of hematology” was established on the internet platform TeleSlide of the company TRIBVN.

Cytological observations are proposed by a series of 4 cases. For the first step the reader is in the same situation as the author: clinical context, CBC and pictures. At this stage, an anonymous vote can propose diagnostic hypothesis and complementary tests, but can also comment on the quality of the presentation.

After 3 weeks, the analysis of the votes and the complete case are registered on line: cytological analysis, complementary tests results and final diagnosis, discussion and references.

Results: 136 cases have been presented since 2003 covering the different types of hemopathies. The cases are mainly derived from cases presented by members of “A.R.C.H.E”, the French group of cellular hematology (GFHC) and the College of hematology.

Each case is viewed on average 1000 times and there are about 30 votes for each case. There are 360 active members registered coming from university hospitals (biologists and students), general hospitals, private laboratories, from France, French speaking countries and North Africa.

There have now been a total of 24000 visits to the site.

Discussion: The increase in the number of active members enrolled in the forum workshop group and in the number of visits to each case over the years show that this type of forum workshop meets the demands of the biologists. Owing to the large numbers of cases, reference pictures can be found by using a key word. These files can also be used as a support for student training.

It is an interactive forum workshop, each member can propose a case.

Conclusion: The significant growth of the forum workshop "Questions of Hematology" over the years reflects the interest shown by the biologists who are concerned with cytological diagnosis in hematology.

Online teaching of inflammatory skin pathology by a French-speaking international university network
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CANADA, FRANCE

Introduction: Developments in technology, web-based teaching and whole slide imaging have broadened the teaching horizon in anatomic pathology. Creating online learning material including many types of media like radiologic images, videos, clinical and macroscopic photographs and whole slides imaging is now accessible to almost every university.

Unfortunately, a major limiting factor to maintain and update the learning material is the amount of work, time and resources needed. In this perspective, a French national university network was initiated in 2011 to build mutualised online teaching pathology modules with clinical cases and tests. This network has been extended to an international level in 2012-2014 (Quebec, Switzerland and Ivory Coast).

Method: One of the first steps of the international project was to build a learning module on inflammatory skin pathology intended for interns and residents of pathology and dermatology. A pathology resident from Quebec spent 6 weeks in France and Switzerland to develop the contents and build the module on an e-learning Moodle platform (http://moodle.sorbonne-paris-cite.fr)
under the supervision of two dermatopathologists (BV, MB). The learning module contains text, interactive clinical cases, tests with feedback, whole slides images (WSI), images and clinical photographs. For that module, the virtual slides are decentralized in 2 universities (Bordeaux and Paris 7). Each university is responsible of its own slide scanning, image storage and online display with virtual slide viewers.

**Results:** The module on inflammatory skin pathology includes more than 50 web pages with French original content, tests and clinical cases, links to over 45 WSI and more than 50 micro and clinical photographs. The whole learning module is currently being revised by four dermatopathologists and two senior pathologists. It will be accessible to interns and residents in spring 2014. The experience and knowledge gained from that work will be transferred to the next international fellowship intern whose work will be aimed at creating lung and breast pathology learning modules.

**Conclusion:** The challenges of sustaining a project of this scope are numerous. The technical aspect of whole-slide imaging and storage needs to be developed by each university or group. The content needs to be regularly updated, completed and its use and existence needs to be promoted by the different actors in pathology. Of the great benefits of that kind of project are the international partnerships and connections that have been established between numerous French-speaking universities and pathologists with the common goals of promoting education in pathology and the use of technology including whole slide imaging.

* The Moodle website is hosted by PRES Sorbonne Paris Cité, and financial supports for hardware have been obtained from UNF3S (http://www.unf3s.org/) and PRES Sorbonne Paris Cité. Financial support for international fellowships has been obtained from CFQCU (http://www.cfqcu.org/).
A5: Technology Advances-1

Confocal Fluorescent Whole Slide Imaging
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Introduction: Fluorescent whole slide imaging became established in the recent years. There are several companies offering products with fluorescent slide scanning capability. Our goal was to build a confocal fluorescent whole slide imager to combine the advantages of confocal microscopy which are minimal background noise, high specificity, optical sectioning with the advantages of whole slide imaging which are seamless imaging of large areas, at high magnification with minimal human interaction.

Material and Methods: For confocal image creation an Aurox Ltd. (Abingdon, UK) aperture correlation spinning disk unit was used. The confocal unit was equipped with four filter blocks, a quad band filter for DAPI, FITC, TRITC and Cy5, and three single band filters for SpectrumOrange, SpectrumGreen and SpectrumAqua. A Pannoramic MIDI fluorescent whole slide imager was modified to accommodate the confocal unit. The confocal unit images a tilted plane relative to the microscope slide. To create seamless digital slides the optical path of the scanner had to be tilted accordingly. A 6 channel Lumencor solid state light engine was used as a light source. The system had an objective changer with two objectives, a Zeiss PlanApochromat 20x, NA 0.8, and a PlanApochromat 40x, NA 1.2 water immersion objective. The scanner was equipped with a custom developed water dispenser system to enable the unattended scanning of 12 slides. For imaging a PCO.edge 5.5 scientific CMOS (Complementary Metal-Oxide Semiconductor) camera was used from PCO AG (Kelheim, Germany). The Pannoramic Scan control software was modified to control the scanner and confocal unit running on a 64 bit, Windows 7 PC. For testing 10 µm thick breast cancer slides were stained with Vysis (Abbott Laboratories. Abbott Park, Illinois) Her-2/neu (SpectrumOrange) / CEP 17 (SpectrumGreen) probe.

Results and Discussion: Twelve FISH slides were scanned with the 40x immersion objective in the DAPI, SpectrumGreen and SpectrumRed channels. The green and orange channels were recorded in 10 layers with 1 µm layer distance and the DAPI channel was recorded in 1 layer. On each slide a 3 x 3 mm rectangle was preselected in the scanner software that included the cancerous area. The exposure time was set manually to 200 ms in each channel. One field of view was 122 x 243 µm. The resolution was 0.16 µm / pixel. The 3 x 3 mm area resulted in 325 fields of views. On average focusing took 3 minutes per slide on the DAPI channel, scanning took 31 minutes and the flat field correction in post processing 7 minutes. The FISH spots were well visible and easy to count on the digital slides.

From the different confocal techniques like point scanning, line scanning, Nipkow disk and aperture correlation we selected the later one because it provides the highest light efficiency and it can be used with wide field illumination. A 6 channel solid state light source for wide field illumination is far more compact and economical compared to a light source composed of 6 lasers.

Conclusion: Confocal whole slide imaging is possible with state of the art components and provides useful results.

Incorporation of neighborhood constraints to Fuzzy C-Means algorithm to improve the spectral histology of human tissue sections by Raman microimaging
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Introduction: Raman microimaging is a label free technology able to probe the biochemical composition of samples. Applied to human normal or cancerous biopsies, it has proved its potential to be a diagnostic aid tool for the pathologist. Unavoidable, the full interpretation of Raman microimages is based on the clustering or classification of the Raman spectra composing these images. However, Raman spectra are corrupted by noise and complex instrumental distortions which are hard to correct. The residual noise and distortions induce a salt-and-pepper spurious classification which makes difficult for the pathologist to interpret the segmented image.

Material and Methods: Three frozen 10µm thick slices were cut with a microtome from normal or cancerous biopsies of human skin or colon. Slices were mounted on a calcium fluoride (CaF₂) window for spectral acquisition performed with a Raman micro-spectrometer using a 785 nm laser
The Raman spectral images were then processed by the Fuzzy C-Means (FCM) algorithm. To overcome the apparition of spurious pixels on the segmented image, an improved version of FCM is proposed in this study. The updating rules of FCM are modified in order to incorporate spatial constraints by considering neighboring spectra.

Results and discussion: Compared to classical FCM, the proposed method compensates the influence of noise and distortions by exploiting the information shared by the neighboring spectra. On the estimated partitions, the different tissue structures of the samples are better defined, localized. The interpretation of the results by a pathologist is thus highly facilitated.

Only three different tissue slices were considered in this work. Future work will be dedicated to the confirmation of the proposed algorithm behavior on a bigger Raman data collection. Furthermore, our method must be compared to the huge number of methods developed in literature for incorporation of spatial constraints in FCM.

Conclusion: Raman microimaging is a promising tool for biochemical investigation of cancerous tissues. However, development of new numerical analysis tools is necessary to make complex Raman data cubes easily interpretable by a non-specialist such as pathologists. In this study, spatial information is added to FCM in order to weight the influence of a pixel by its neighbours.

Prostate cancer assessment using stain-free, three dimensional, quantitative imaging (Genesis™200) - a comparison to standard histology on whole-mount sections

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Introduction: Prostate cancer (PC) is the most frequently diagnosed cancer in males. Radical prostatectomy is considered a standard treatment in clinically localized PC. Prognostic factors for disease recurrence and progression to a metastatic state include Gleason-score, cancer volume and margin status as well as nodal status. Whole-mount section based assessment of the entire prostate is considered the current gold-standard. However, prostate histopathology is associated with significant inter-observer variability. Hence, there is an urgent need for more standardized investigation. Herein, we present our initial experience with stain-free, three dimensional, quantitative imaging (Genesis™200) in the assessment of PC.

Materials and Methods: Genesis™200 is the first in the world to provide stain-free, three dimensional, quantitative imaging for visualizing and staging fibrosis and connective tissue alterations. This provides critical information which is currently not available to pathologists with existing stain-based imaging techniques. Whole-mount sections (4µm) 3 slides were taken from radical prostatectomy specimens. Standard HE-assessment was done by an uro-pathologist. The sections were digitalized and all cancer foci were graded according to Gleason and marked. Every other section remained unstained. These were analyzed using the Genesis™200 system and the results were compared to standard histology.

Results: A total of 2 slides were analyzed. Prostatic micro-anatomy could be visualized in all including the urethra, the transitional zone as well the peripheral zone. A characteristic fibrosis pattern could be demonstrated in and around PC foci representing a cancer-specific microenvironment. Positive margins could be demonstrated as well. Correlation of presumed cancer-foci on Genesis™200-imaging to HE was concordant in all cases.

Conclusion: Genesis™200 can be reliably applied to non-stained whole-mount sections of the human prostate. PC-foci could be demonstrated and confirmed. Moreover, margin-status assessment could be done. Further, validation studies are required applying Genesis™200 to biopsy cores as well as to intraoperative frozen section analysis. This novel technology has the potential to become a pivotal tool in radically changing the future of PC-histopathology.

Digital pathology with the Fourier ptychographic microscope

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Fourier ptychographic microscopy (FPM) is a recently introduced method of acquiring high-resolution (0.37² µm²), wide field of view (120 mm²) giga-pixel images of histology samples. The FPM procedure begins by acquiring a sequence of low-resolution images of a sample under variable-angle illumination from a fixed set of light-emitting diodes (LEDs). It then combines this sequence of images using a novel phase retrieval algorithm to improve the employed microscope’s resolution beyond the limit defined by its optical
elements. Any optical aberrations within the microscope objective may likewise be automatically accounted for during computational post-processing to offer sharp reconstructions across the entire image field-of-view (FOV).

Here, we first describe how FPM can improve the resolution of histology samples beyond what a typical microscope objective offers over the same FOV. Second, we show that FPM may also record the optical phase information contained within a thin sample. Phase may be used to digitally refocus portions of the image that are not clear, or can lead to an estimation of the sample’s scattering coefficient, reduced scattering coefficient and anisotropy factor. Experimentally generated maps of these optical parameters are included to support the conclusion that FPM can potentially aid pathologists in digitally extracting as much optical information as possible from a given histology sample.

**PatternQuant supported Image Analysis for IHC quantification**

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**Hungary**

**Background:** In the era of targeted therapy pathology report provides inevitable predictive data for therapeutic decisions. In case of breast cancers nuclear estrogen/progesteron (ER/PR) receptor expression is evaluated according to Allred-score spanning 0-8, while membranous HER2 expression is evaluated in a 4-tiered score. Both score has crucial role in determining further treatment requiring high standards and quality assurance in pathological reporting. Besides the easy and truecut cases, where classification into the positive and negative classes is straightforward, more cases lay in the borderline category where classification is problematic; still the therapy is decided upon the expression results of the pathology reports raising huge responsibility for the pathologists’ eye. We assume that high fidelity, reliable and reproducible reports and standardization of evaluation can be achieved with digital pathology methods resulting in more effective patient selection. Therefore we compared and validated digital and manual evaluation with automated evaluation, with an emphasis on clinical relevance.

**Findings:** 186 breast cancer cases were evaluated immunohistochemically for nuclear hormone receptor and membranous HER2 expression semi-quantitatively on glass and digitized slides by 3 pathologists and by an automated method using PatternQuant recognition tool of the Pannoramic platform (3DHistech, Budapest, Hungary). These data were then compared by calculating Cohen’s kappa (CK) and Quadratic weighted kappa (QWK) in each interobserver (between pathologists) and intermethod (manual versus semi-automated) setting. Semi-quantitative HER2 interobserver agreement reached CK: 0.712-0.779, with QWK: 0.925-0.942. Digital reading of HER2 interobserver CK: 0.698-0.722, with QWK: 0.912-0.916. Intermethod HER2 CK: 0.579-0.820, with QWK: 0.876-0.951. Semi-quantitative nuclear interobserver agreement with estrogen reached CK: 0.456-0.645; QWK: 0.917-0.956 and with progesteron reached CK: 0.496-0.642; QWK: 0.924-0.955. Digital reading of nuclear positivity with estrogen reached interobserver CK ER: 0.532-0.633, QWK ER: 0.930-0.961, and with progesteron CK: 0.618-0.640 and QWK: 0.943-0.962. Estrogen intermethod CK: 0.329-0.767, QWK: 0.894-0.973, progesteron intermethod CK: 0.432-0.781; QWK: 0.904-0.982.

**Conclusion:** According to our study, the evaluation of membranous HER2 immunostaining on glass and digitized slides resulted the same data with the latter method offering a more convenient and flexible method, which gains even more importance with recent changes in HER2-guidelines. Nuclear immunostainings’ evaluation was similar on digitized and on glass slides, whereas digital reading had improved CK and QWK-values, especially with PR-immunoslides. Furthermore, automated evaluation with PatternQuant significantly reduced evaluation time offering an effective help for standardized and achieved evaluation of unequivocal cases, which before have raised diagnostic problems for the "tired" eyes of the pathologists. As expensive anti-HER2-therapy also has cardiovascular side effects, while on the other hand the wide range of Allred-index poses questions of reproducibility, standardization by digital evaluation might improve patient selection for more effective targeted therapy.
Standards and Recommendations for Digital Pathology: Image Selection and Annotation.
FRANCE, SINGAPORE

Introduction: Pathologists are shifting from the ancillary optic microscopic observation towards digital pathology. Concerning review of series of medical cases the method includes paper form(s) or file(s) adapted to the aim of the slide review. Digital imaging in pathology has simplified the procedure for case sharing either by uploading files or sharing the cases on a web site. As our practice evolves it seems that standardizing the link between the annotation and the spot, image, region of interest on the virtual slide needs some formalization for optimal work.

Material and Methods: Relation between image and annotation is mandatory for: word research, semantic, libraries, comments, legends, codification, algorithms and contextualization of the whole procedure. We applied a protocol for such procedure that has been progressively included in our practice. We have a word glossary and technical rule for: denomination and localization of the parts of the virtual slide that is visualized.

Results and Discussion: Hand delimited parts of the slide are called territories with adapted qualification/denomination, and are included in the list of descriptive items (template). The word region has been considered as generic. The detailed items that need to be pointed for further discussion or investigation are looked for and pointed in pre-defined frames. The frames are square, ranging from size 10 (more or less X10 of our microscopes), it includes 4 frames size 20, which each include 4 frames size 40. The annotations are then related to a territory, with or without frames, the annotations/comments can be global for the delimited part of the slide in the frame. Coming to detailed semiology further annotation needs precision and then each point is spotted at a higher 40 frame. As an example: pathologists may quote the area in a 20 frame as high or low for atypia but the support of such comment can be detailed and traced pointing and annotating the relying items such as nuclear size, nucleoli, nuclear shape point by point with each point commented and spotted at multiple 40 frames within the commented area. The very detailed spot/annotation need to be precisely pointed. We use then a “window”, small adapted to the object of interest so that the detail or interest is at evidence the center of the smallest image/icone of or Imaging Sytem.

Conclusion: digital pathology will hopefully facilitate the communication and investigation amongst pathologists.

Standardization is needed to collaborate using whole slide images
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Introduction: Slide scanners are becoming increasingly popular because current scanners are fast enough and produce good enough quality images for clinical use (despite the current absence of FDA approval). In the Netherlands the use of digital pathology is quickly increasing, a few labs even started doing digital diagnostics. To use Whole Slide Images (WSI) for consultation it is not only needed to share the images, but also relevant clinical information and have the whole process integrated into the regular workflow. To be able to create cross-laboratory workflows where systems from different vendors are involved, standardization is highly needed.

Materials and Methods: To support an efficient workflow parties involved in the consultation process must implement internationally available standards that support these kinds of use cases. In this way it is possible to exchange data between systems from different vendors. These standards are available and described by the international organization Integrating the Healthcare Enterprise (IHE), where healthcare providers and vendors are involved. IHE describes in so called “profiles” solutions for real world health care use cases based on available standards like DICOM, HL7, web services, etc.

In the Netherlands there has been a national database of pathology reports since the seventies. This database is maintained by PALGA to which all pathology laboratories are committed to send their reports. In 2013 an initiative was started by about 20 pathology laboratories who joined together to discuss opportunities to adopt relevant standards to make collaboration between laboratories possible. This initiative has the goal to setup a national network to exchange those images. Note: PALGA is also involved in this initiative.
Looking to developments in the radiology domain, the solutions described by IHE can be used when implemented by vendors active in this market. One such profile is XDS-I, Cross-enterprise Document Sharing for Imaging. When those standards are not yet supported it is possible to use middleware software to make the translation between proprietary internal data and the communication to external laboratories. The use of standards supports easier integration of digital pathology with the already available image management software (e.g. PACS, Vendor Neutral Archives).

**Conclusion:** Digital pathology in general is making a lot of progress to become a mature and more generally accepted technique amongst pathology laboratories. The next step will be to use Whole Slide Images in cross-enterprise communication, not only for intercollegial consultation, but also for revisions, tumor boards, etc. The only way to be able to create a coherent workflow is when all participating laboratories have equipment that is able to communicate based on standards (like the ones described by IHE in the XDS-i profile). The current status of the adoption of digital pathology is the right moment to start using standards. The Dutch initiative of 20 laboratories working together is a good example and is very promising because it can eventually lead to a national network for pathology image exchange for e.g. consultation, integrated with the already existing national report database.

**Convergence in Digital Pathology data sharing: A standard recommendation for digital pathology information web-interface**

**Yves Sucaet, Win Waelput**

**Belgium**

**Introduction:** A recommendation on Digital Pathology Information Web-Services (DPIWS) standard is presented, with respect to the specific characteristics of the informative content of discourse. The recommendation establishes a common software interface for the exchange of Digital Pathology (DP) images and annotations through the web, independently of the storage, encoding and internal handling details. The proposed structure is implemented and tested through the Pathomation™ cloud environment. One of the major obstacles in establishing and adopting effective telepathology processes overtime has been their lack of information brokerage standardization [1].

DP imagery and annotations distribution is to-date partially covered by specific portions of the Digital Imaging and Communications in Medicine (DICOM) and the Open Microscopy Environment (OME) standards. In addition, DP information sharing is found to bear significant similarities to other disciplines, the distribution of which has been highly standardized since decades. The proposed recommendation (DPIWS) delivers a standard web interface definition allowing requests for pathology information elements handling and sharing across the web, through platform-independent and image format-agnostic calls.

**Materials and Methods:** DPIWS comes as an independent recommendation. It strictly conforms to and expands the DICOM Standard [2], with respect to the OME [3] and the Open Geospatial Consortium (OGC) Web Map Service (WMS) & Web Feature Service (WFS) Standard [4].

DP images are partially covered by the DICOM 2011-Part 3, A32.2 and A32.8. In addition, generic URL requests for retrieving a DICOM Visual Light image are defined under DICOM 2011-Part 18. The response though is a single, standard encoding image with all annotations being rendered (“burned”) on the image; no method for requesting and handling annotations in a form other than image is identified.

Annotations on microscopy images are on the other hand exhaustively covered by the OME. 2-D and 3-D Regions of Interest may be defined and treated and a series of annotation elements are supported; this makes OME an appropriate tool for DP annotations handling.

In addition, OGC through WMS & WFS, effectively defines the web-handling of multi-layered, complex raster images and vector data, making it the ideal interface: a. To uniformly query and retrieve composite DP images of superimposed multi-resolution raster, vector and textual annotation layers and b. To uniformly share the results across the web in spite of the complexity and structure of the content.

**Results and discussion:** The proposed recommendation is an interdisciplinary standard, designed to address the specificities of Digital Pathology information brokerage.

For this: a. it conforms to DICOM: images treated internally and served by DICOM compliant systems may distribute content to DPIWS compliant clients “as-is”. b: it adopts the OME annotation structure to form handling requests based on the well-structured XML OME annotations and c: it adopts and extends the WFS
interface for DP information web-handling and delivery.
Building DPIWS compliant services will eventually eliminate the risk of long-term vendor content locking, boosting the digital transition in the field of pathology.

**Conclusion:** A standard for DP information brokerage across the web is defined, implemented and tested in real-life cloud-based distributed operating environment.

**Digital immunohistochemistry platform for the staining variation monitoring based on integration of image and statistical analyses with laboratory information system**

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**Background:** Digital immunohistochemistry (IHC) is one of the most promising applications brought by new generation image analysis (IA). While conventional IHC staining quality is monitored by semi-quantitative visual evaluation of tissue controls, IA may require more sensitive measurement. We designed an automated system to digitally monitor IHC multi-tissue controls, based on SQL-level integration of laboratory information system with image and statistical analysis tools.

**Methods:** Consecutive sections of TMA containing 10 cores of breast cancer tissue were used as tissue controls in routine Ki67 IHC testing. Ventana slide label barcode ID was sent to the LIS to register the serial section sequence. The slides were stained and scanned (Aperio ScanScope XT), IA was performed by the Aperio/Leica Colocalization and Genie Classifier/Nuclear algorithms. SQL-based integration ensured automated statistical analysis of the IA data by the SAS Enterprise Guide project. Factor analysis and plot visualizations were performed to explore slide-to-slide variation of the Ki67 IHC staining results in the control tissue.

**Results:** Slide-to-slide intra-core IHC staining analysis revealed rather significant variation of the variables reflecting the sample size, while Brown and Blue Intensity were relatively stable. To further investigate this variation, the IA results from the 10 cores were aggregated to minimize tissue-related variance. Factor analysis revealed association between the variables reflecting the sample size detected by IA and Blue Intensity. Since the main feature to be extracted from the tissue controls was staining intensity, we further explored the variation of the intensity variables in the individual cores. MeanBrownBlue Intensity ((Brown+Blue)/2) and DiffBrownBlue Intensity (Brown-Blue) were introduced to better contrast the absolute intensity and the colour balance variation in each core; relevant factor scores were extracted. Finally, tissue-related factors of IHC staining variance were explored in the individual tissue cores.

**Conclusions:** Our solution enabled to monitor staining of IHC multi-tissue controls by the means of IA, followed by automated statistical analysis, integrated into the laboratory workflow. We found that, even in consecutive serial tissue sections, tissue-related factors affected the IHC IA results; meanwhile, less intense blue counterstain was associated with less amount of tissue, detected by the IA tools.
An algorithm for reducing stain variability in scanned histological slides

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THE NETHERLANDS

Introduction: With the advent of digital pathology, whole slide imaging is now feasible for larger numbers of microscopic slides at high resolution. Besides enabling remote diagnosis and facilitating diagnostic workflow, this also yields opportunities for computer aided detection and diagnosis (CAD). An important prerequisite for introduction of CAD algorithms is robustness to variations in specimen quality. It is well known that especially the traditional staining procedures in pathology (e.g. hematoxylin and eosin; H&E) suffer from large inter- and intra-laboratory variation. This study investigates the relative contribution of several factors to the color variation of H&E stained slides. The study also proposes an algorithm for color standardization of histology slides.

Methods: The image data used in this study originate from a set of 45 digitized H&E stained histopathology slides of sentinel lymph nodes from three different patients. Slides were produced by serial sectioning and were subsequently stained in three different laboratories on five different week days. A number of microscopic measurement fields were then digitized using a CCD RGB camera mounted on a light microscope. The variation of the pixels absorbing mostly the Hematoxylin stain (i.e. pixels within nuclei) was statistically measured using the hue-saturation-density (HSD) color model. The importance of three potential factors that contribute to staining variations (patient, staining laboratory and staining day of the week) were studied. The algorithm for standardization of the histology slides is based on an initial clustering of the image pixels into two classes having different tissue absorption characteristics for different stains (H and E). The color distribution for each dye is standardized by aligning the 2D histogram of color distribution in the HSD model.

Results: Experimental results demonstrate that staining protocols in different laboratories and staining on different days of the week are the major factors causing color variations in histopathological images. In contrast, differences between slides from different patients in a single staining batch are negligible. Qualitative evaluation of the proposed standardization algorithm shows that color constancy of the standardized images is highly improved. Quantitative evaluation demonstrates that the algorithm outperforms competing methods.

Conclusion: Results from the present study demonstrate that staining variations, which may potentially hamper usefulness of computer assisted analysis of histopathological images, can be reduced considerably by applying the proposed algorithm. In cases where standardization of the staining procedure is not possible, the algorithm will still enable application of a single CAD procedure for slides produced by different laboratories and scanners. Also, the algorithm may facilitate the pathological evaluation in multicenter studies by removing differences between slides from different clinical sites, removing a possible confounder.

Assessment of chromosome instability predicts progressive potential of oral premalignancies


THE NETHERLANDS

Introduction: Reducing the incidence of oral squamous cell carcinoma (OSCC) is hampered by our inability to assess the progressive potential of oral premalignancies. Histopathological examination of leukoplakia in the oral cavity has been shown to be insufficiently accurate to predict malignant transformation. This may lead to both over- and under-treatment of patients. The present study aims to assess predictive power of detection of chromosome instability (CI) for predicting progression of oral leukoplakia, as well as for monitoring premalignant lesions over time. CI is studied using both DNA image cytometry (ICM) and fluorescent in situ hybridization (FISH).

Materials and Methods: Paraffin-embedded tissue of oral leukoplakia (n=102) were taken from our archives and included in this study. Patient follow-up data were collected and the histopathological diagnosis was revised. DNA ICM and dual target FISH for chromosomes 1 and 7 were performed on biopsy specimens obtained at the time of the first diagnosis of leukoplakia, on possible subsequent premalignancies and on the specimens from eventually developed carcinomas. Nuclei obtained from 50µm sections were isolated, stained with Schiff-Feulgen and the
DNA content measured. For FISH, 4µm deparaffinized sections were hybridized with specific centromere probes for chromosome 1 and 7. Uni- and multivariate Cox regression were used to study the relationship between CI and malignant progression of premalignant lesions.

**Results and Discussion:** Both detection methods were found to yield prognostic information independent of the histological diagnosis. Presence of CI yielded hazard ratios of 7.2 (ICM) and 6.8 (FISH), showing it to be a strong independent prognosticator. Also, we found that especially ICM is a suitable tool for monitoring premalignant lesions over time. Almost all aneuploid malignancies resulted from aneuploid precursor lesions and almost all diploid carcinoma had a diploid precursor lesion. Combining histopathology and CI enables subdivision of patients into three risk groups, with strongly increasing probability of malignant progression: 1) diploid low grade, 2) diploid high grade or aneuploid low grade and 3) aneuploid high grade. Comparing the first and third patient group yielded a hazard ratio of 21.9.

**Conclusion:** CI detection seems a reliable method for risk assessment of oral premalignancies and its application may contribute to a better risk-counseling and appropriate treatment regimen or watchful-waiting approach of patients.
A7: Models, Data Mining and Knowledge Formalization in Pathology

Fractals, structural entropy and image content analysis in histopathology
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Introduction: Diagnostic surgical pathology, i.e. tissue–based diagnosis requires microscopic images. Digital image acquisition and virtual microscopy have led to separately define and apply image content analysis (ICA) in computerized assistance of digital pathology diagnosis. Herein we discuss some basic aspects of ICA in relationship to included/aimed external information and image presentation.

Definition and Performance: By definition, ICA is the computerized search for, identification of, and evaluation of features of microscopic image areas that are highly characteristic to derive the corresponding diagnosis. It results in the determination of so–called regions of interest (ROI) and herein performed measurements. In practice two different algorithms can be distinguished, a) interactive human and b) automated machine action. In both algorithms, external image information is assigned at different information levels to image features at different magnifications. In a second step an “invert” function is calculated that transforms the evaluated image features to the external diagnosis. Evaluated image features can include external information too (objects, structures) or be completely independent (pixel–based primitives and textures). Feature evaluation algorithms can be applied to original or transformed (for example Fourier, Fractals, Gradient, Hugh, Laplace, etc.) images. Feature parameters are either individual expressions such as size, gray value content and derived functions (form factor, moment, etc.) or neighbourhood dependent (entropy, structural entropy, image primitives). Fractal and Fourier analyses are mainly performed in high magnification images (nuclei). Voronoi’s tessellation is the most frequently applied neighbourhood condition.

Details and Results of Applied Algorithms: The developed and tested algorithms work with fixed and flexible sizes of areas applying texture, object and structure analysis as well as fractals and Fourier analysis. Graph theory approach with minimum spanning tree calculation of conventional and structural entropy as well as evaluation of object related features were tested. The results were validated by learning and test sets of different diagnoses in a broad set of tissues. Crude diagnoses could be derived automatically from the selected ROIs with a specificity and sensitivity > 95%. The preference of neighbourhood analysis in low magnification images and the potential value of Fourier and fractal analysis in high magnification images is discussed.

Conclusion: The described theory of ICA includes different algorithms which present with different levels of external information. In addition to image transformation, such as fractals or Fourier, neighbourhood analysis and associated features such as entropy and structural entropy can provide reliable assignment and “inverse” functions to either describe or evaluate ICA.

Towards Ontology-Driven High-Content Image Analysis. An Operational Instantiation for Mitosis Detection for Digital Histopathology
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Our study concerns a novel operational framework related to symbolic cognitive vision approach, emerging from the Cognitive Microscopy (MICO) ANR TecSan initiative. MICO aims at supporting the evolution towards clinical digital pathology, by elaborating procedures allowing the emergence of future clinical routines. We designed and prototype a decision support system, instantiated for breast cancer grading, in histopathology. Taking benefit of the virtual microscopy environment as of state-of-the-art multi-scale medical image analysis technologies, we support high-level decisions taken by pathologists, while helping them to avoid automatically tedious and time-consuming quantification tasks. Integrating ontologies and reasoning in confluence with modular imaging algorithms allows the emergence of new clinical protocols, allowing a deep analysis of the cancer evolution (multiple slides systematic analysis for the same sample), better (second opinion always available) quality cancer gradation, fast available technologies (extemporaneous analysis) and new complete and reliable cancer gradation strategies. The key concept behind MICO project is the role
of the semantics “at the helm” of the exploration process. All the decisions being taken into a glass box, semantic and formal world, MICO represents a knowledge-driven platform for histopathology. The core of this initiative is knowledge representation and reasoning. Histopathology experts’ knowledge and strategies are so used to address image processing algorithms computational limitations. In this sense, hard-coded knowledge, semantic and usability gaps are to be reduced by a leading, active role of reasoning and of semantic approaches. This represents a promising way to solve decision reproducibility and traceability issues in histopathology field, while increasing platform flexibility and pathologist acceptance, the one always having the last word and responsibility in the healthcare process. Last, but not least, the generic approach issued from the proposed instantiation is applicable for number of additional challenges, related, for example, to cytology, biology, molecular imaging and, in general, to high-content images analysis approaches.

Fourier parameters and fractal features distinguish between keloids and hypertrophic scars

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Introduction: Hypertrophic scars and keloids are two different clinical entities with different prognosis, which are difficult to distinguish by histologic examination. The aim of the study was to introduce computer assisted texture analysis of histologic slides in order to separate both entities comparing programs based on Fourier analysis with those based on fractal feature extraction.

Material and Methods: Cases were selected from our files. The final diagnosis had always been based on histological and clinical features. Random images from histologic slides stained according to Masson were digitalized. Grayvalue-transformed images were analyzed by inhouse programs based on FFT (fast Fourier transformed) images, Haralick features or fractal characteristics. We introduced new variables derived from the FFT images introducing FFT vectors for each pixel (length determined by its luminance and the direction defined by the position relative to the center) for different frequency ranges.

Results and Discussion: Using discriminante analyses, it was possible to classify correctly more than 80% of the cases by texture parameter combinations including FFT or Haralick features. A similar degree of correct diagnoses was achieved by analyses based on fractal characteristics.

Images of keloids are generally more homogeneous in terms of the gray levels. Both for high and low spatial frequencies, keloids showed more pronounced anisotropy.

Conclusion: Texture analysis parameters based on FFT or fractal characteristics can be helpful for the objective differentiation between keloids and hypertrophic scars.

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Heterogeneity assessment of histological tissue sections in whole slide images

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Introduction: Computerized image analyses (IA) provide quantitative and repeatable object measurements by means of methods such as segmentation, indexation or classification. IA can contribute to more accurate determination of prognostic histological factors by pathologists working on whole slide images (WSI) from histological sections. A main challenge WSI technology presents is the size of data to be processed which is very large. In parallel, the aggressiveness of a cancer could be linked to morphological and architectural changes that can be observed in tissue structure and so be characterized by the object distribution on the slide, by cross-relations between objects and by the texture. This kind of information could contribute to evaluate a well-known concept: heterogeneity. Frequently addressed in signal processing but more rarely in the field of imaging, the objective is to propose a framework for measuring tissue heterogeneity in WSI of breast cancer images. The key idea is to not rely on segmentation of individual structures to characterize heterogeneity but to make use of patch classification.

Material and Methods: WSI come from histological sections of breast cancer stained according to the Ki67 protocol and Hematoxylin-Eosin-Saffron protocol (HES), then split in connected blocks of size 50x50 pixels called “patches”. Each patch is associated with a numerical signature expressed as a large dimension feature vector, embedding statistical measures and texture parameters from color
Cell Words: A Novel Paradigm for Modeling Cells in Histopathological Images
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Introduction: Detection and classification of cells in histological images is a challenging task because of the large intra-class variation in the visual appearance of various types of biological cells. The problem becomes even challenging because of issues like occlusion and degree of contact/overlap with other cells. Consider for example mitosis, which is a process during which a cell duplicates into two daughter cells after undergoing various complex transformations. Detection of mitotic cells (MCs) is a challenging task because of the large intra-class variation in the visual appearance of MCs. Additionally, if standard hematoxylin and eosin (H&E) staining is used, which stains chromatin rich structures, such as nucleus, apoptotic and MCs dark blue, it becomes extremely challenging to detect the MCs given the fact that former two are densely localized in the tissue sections. Traditional approaches to detection of MCs extract specific characteristics (e.g. size, compactness etc.) from an object to perform this task, which may not have requisite discriminability to separate objects with subtle appearance differences. In this paper, we propose a novel paradigm (termed as Cell Words) for modeling the appearance, which includes color, shape, size, texture and context in a unified manner using Discriminative Dictionary Learning (DDL). The proposed framework is capable of distinguishing mitotic cells from non-mitotic cells (apoptotic, necrotic, epithelial) in breast histology images with high accuracy.

Methods: We propose a DDL paradigm to model the visual appearance (shape, texture, intensity, color and context) of cells in histopathological images. Histopathological image patches having a cell in the center are used to learn a discriminative dictionary, where dictionary atoms have correspondence to the class labels. The dictionary generated as a result consists of discriminative ‘cell words’ belonging to both mitotic and non-mitotic classes. The proposed DDL method considers an average of the cost function, consisting of total reconstruction error as well as inter-class and intra-class reconstruction error. By using the average of the cost function, the proposed DDL method is insensitive to class imbalance, a classical problem which lower the performance of other learning algorithms. We also use variable number of dictionary atoms to capture the variation in majority class while keeping the dictionary compact. Results and Discussion: We evaluate the proposed method on the publicly available MITOS dataset. Based on the criteria provided by the 2012 International Conference on Pattern Recognition (ICPR) Mitosis Detection Contest, the proposed method yields precision of 0.67, recall of 0.81, and F1-score of 0.73. Out of 14 competitive methods, the proposed method is only second to the deep-neural-network based algorithm which provides...
F1-score of 0.78. However, the deep-neural-network based algorithm requires much higher computational time and resources than the proposed method. **Conclusion:** We presented here an efficient paradigm for detection of cells/nuclei in hispathological images using DDL framework. In particular, we demonstrated the proposed framework on the detection of MCs in breast histopathological images. In comparison with the state-of-the-art in literature, the proposed framework demonstrated encouraging results on MITOS dataset.
Quantifying histopathological features and novel phenes through image analysis to stratify colorectal cancer patients

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Introduction: Surgical resection is usually considered to be a curative procedure for Dukes A and B colorectal cancer (CRC) patients. Adjuvant therapy is therefore not routinely administered; however, 5% of Dukes A and 15-20% of Dukes B patients experience disease progression and poor prognosis. It is therefore imperative to identify these high risk patients. Tumour budding (TB), lymphatic vessel density (LVD) and lymphatic vessel invasion (LVI) have shown to be correlated to poor prognosis although under-recognition, lack of standardisation and observer variability mean they are not routinely reported in the clinic. We demonstrate the robust quantification of the three histopathological factors, at the invasive front, through digital pathology coupled with image analysis, which would permit standardisation across institutes.

The colorectal invasive microenvironment is a complex ecology consisting of multiple morphologies and phenomes, making it difficult to manually quantify. We performed unbiased multi-parametric image analysis across the invasive front in an attempt to capture novel prognostic phenes within this complex microenvironment. These phenes are compiled into a prognostic fingerprint which allows the identification of high risk CRC patients independent of Dukes staging.

Materials and Methods: A multiplexed immunofluorescence stain consisting of pan-cytokeratin, D2-40 and DAPI was utilised to identify cancer epithelium, lymphatic vessels and nuclei, respectively, across the invasive front of tissue sections taken from 50 CRC patients (Dukes A, n=13; Dukes B, n=29; Dukes C, n=8). Patient follow up was up to 15 years. Each stained tissue section was analysed utilising a single image analysis algorithm to quantify TB, LVD, and LVI. Furthermore 123 features were automatically extracted from the image to create a prognostic phenotype fingerprint in order to cluster patients, after data reduction by multi-dimensional scaling, into high and low risk of poor outcome.

Results: The automatic quantification of TB, LVD and LVI showed all three to be significantly correlated with poor outcome and that upon multivariable analysis LVI (HR =6.08; 95% CI, 1.17-31.41) was seen to be an independent prognostic factor. Plotting of the multi-parametric prognostic fingerprint, captured from the invasive front, showed a clear divide between Dukes B patients with good and poor prognosis.

Conclusion: We demonstrate methodology through image analysis which can standardised the quantification of TB, LVD and LVI from a single tissue section. We suggest this technology is capable of stratifying high risk CRC subpopulations and show the three histopathological features to be of prognostic significance. We also provide methodology to capture novel phenes from the complex invasive microenvironment and compile these into a prognostic fingerprint with the ability to identify high risk CRC patients independently of Dukes staging.

Nucleoli Detection using the Cascade Detector

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Introduction: Digital Pathology is a new area of research incorporating image processing and machine learning. High resolution imaging by virtual microscopy leads to new ways and algorithms to analyze the images. Our work focuses on cancer detection in specimens/slides related to prostate, renal and breast cancer. General process of observing a slide involves finding pre-defined patterns of cancer.

Material and Methods: The slides are stained by Hematoxylin and Eosin (H&E) stain. Parts of high resolution images of slides are cropped out for analysis. We use variants of Histogram of oriented gradients (HOG) as feature descriptors. These histogram features are used for classification by either the Support Vector Machines or a boosting algorithm like Adaboost. A simple classifier based on logistic regression is also used. A classifier using an improved version of Independent Component Analysis (ICA) was also used in our algorithm.

The large number of background pixels leads to a large number of false positives although the classifiers can be accurate. To counter this issue we use a cascade of classifiers to reduce the overall false positives in the final output.
Results and Discussion: Average precision is defined as area under the Precision-Recall curve. Higher the average precision in testing, better is the classifier. Maximum and minimum values for average precision are 1 and 0. We compare the performance of 3 cascades by using the average precision of classifier at each level. First cascade uses Logistic Regression classifier only, second cascade uses XICA classifier only and third uses different classifiers in its 20 levels. For the first cascade with Logistic Regression classifiers, the average precision changes from 0.19 at first level to 0.136 at the final level. For the second cascade with XICA classifiers, the average precision changes from 0.06 at first level to 0.05 at the final level. For the third cascade with different classifiers (mixed), the average precision changes from 0.192 at first level to 0.329 at the final level. In the cascade with same type of classifier the average precision tends to decrease with level. At each level all the false positive samples predicted by classifier at previous level are used as negative samples. This leads to lower accuracy in higher levels.

Conclusion: Mixed classifiers cascade show better testing accuracy. Logistic Regression classifier is not very accurate given the fact that it uses simple features made of pixel intensities. A cascade’s performance at its first level is equivalent to performance of a single classifier. In case of mixed classifier cascade average precision of classifier at the final level is better that of at the first level. Hence, using a cascade of classifiers in place of single classifier gives us better performance and accuracy.

Automated Mitosis Detection in Color and Multi-spectral High-Content Images in Histopathology: Application to Breast Cancer Grading in Digital Pathology
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Introduction: Digital pathology represents one of the major and challenging evolutions in modern medicine. Pathological exams constitute not only the gold standard in most of medical protocols, but also play a critical and legal role in the diagnosis process. Diagnosing a disease after manually analyzing numerous biopsy slides represents a labor-intensive work for pathologists. Thanks to the recent advances in digital histopathology, the recognition of histological tissue patterns in a high-content Whole Slide Image (WSI) has the potential to provide valuable assistance to the pathologist in his daily practice. Histopathological classification and grading of biopsy samples provide valuable prognostic information that could be used for diagnosis and treatment support. Nottingham grading system is the standard for breast cancer histopathology; first framework for color dataset and second framework for multispectral dataset. The main contributions of these frameworks are six folds. First, we analyse the statistical and morphological information concerning mitotic cells on different color channels of various color models that improve the mitosis detection in color datasets (Aperio and Hamamatsu scanners). Second, three different methods for spectral bands (SBs) selection for Multispectral dataset including relative spectral absorption of different tissue components, spectral absorption of H&E stains and minimum Redundancy Maximum Relevance (mRMR) technique. Third, we compute features containing pixel, texture and morphological information on selected color channels / spectral bands, which leverage discriminant information for mitosis classification on color / multispectral datasets. Four, we study oversampling methods to increase the number of instances of the minority class (mitosis) by interpolating between several minority class examples that lie together, which make classification more robust. Five, we perform a comprehensive study on region and patch based features for mitosis classification. Six, we perform an extensive investigation of classifiers and inference of the best one for mitosis classification.

Results and Discussion: The evaluation of these frameworks is done in MICO (COgnitive Microscopy, ANR TecSan project) platform prototyping initiative. We thus tested our proposed frameworks on MITOS international contest dataset initiated by this project. For the color framework, we manage to rank second during the contest. Furthermore, our multispectral framework outperforms significantly the top methods presented during the contest. Finally, our
frameworks allow us reaching the same level of accuracy in mitosis detection on brightlight as multispectral datasets, a promising result on the way to clinical evaluation and routine.

**Localization of Luminal Epithelium Edge in Digital Histopathology Images of IHC Stained Slides of Endometrial Biopsies**

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**Introduction**

Uterine Natural Killer (uNK) cells are immune cells found in the human female uterus lining. Normally, these cells make up no more than 5% of all cells in the womb lining. Abnormally high numbers of uNK cells in the uterus lead women to suffer from recurrent miscarriages. Computer-assisted diagnosis of recurrent miscarriage due to over-presence of uNK cells can be made by calculating the ratio of uNK cells to stromal cells in histopathology images of endometrial biopsy stained with Haematoxylin and CD56 (which stains uNK cells brown when used with DAB staining). This process is compounded by the fact that the luminal epithelial edge should be removed and cells on the edge should not be counted as part of the diagnostic process. In this paper, we present a complete solution for detecting the stromal and uNK cells and also for localising the luminal epithelium edge of endometrial biopsy samples.

**Materials and Methods**

The sample images for evaluating our proposed solution are high power fields (HPFs) cropped from digitised images of endometrial biopsy slides stained with Haematoxylin (for stromal cell nuclei) and DAB (for uNK cell nuclei). The proposed solution consists of 2 steps: (1) detecting stromal and uNK cell nuclei and (2) localisation of the luminal epithelium edge.

1. Detection of Stromal and uNK Cells

   We first separate the input image into two stain channels: Haematoxylin and DAB, using the Ruifrok & Johnston method. The Haematoxylin channel is used to detect stromal cell nuclei and the DAB channel is used to detect uNK cell nuclei. Stromal cell nuclei is detected using a modified version of the Local Isotropic Phase Symmetry Measure (LIPSyM) method. We also propose an adaptive background removal method to significantly ease the difficulty in segmentation of uNK cell nuclei regions.

2. Localisation of Luminal Epithelium Edge

   Luminal epithelium edge is the boundary of a tissue region, which is made up by a layer of dense epithelial cell nuclei. Therefore, epithelial cell nuclei can be used to localise luminal epithelium. We propose a novel approach that identifies LIPSyM detections corresponding to epithelial cell nuclei based on the alpha-shape algorithm and localises the luminal epithelium edge via fitting a curve to these detections using a cubic B-Spline.

**Results and Discussion**

We evaluated our solution against expert hand-marked ground truth images. The results show the high accuracy and the robust performance of our proposed solution. In addition, our solution is efficient in term of running speed compared with a commercial cloud computing based software named VIS developed by Visiopharm.

**Conclusions**

In this paper, we proposed an automatic solution for detecting stromal and uNK cell nuclei and localising luminal epithelium edge of endometrial biopsy slides in H&DAB stained digital histopathology image. The solution performs with high accuracy and fast speed on high resolution sample images and is ready to be extended for use with whole slide images.
**Single Cell Segmentation with Watersheds on Highly Multiplexed Images**  
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SWITZERLAND

**Introduction:** Single cell protein expression analysis requires appropriate single cell segmentation in cell images. Conventional immunohistochemistry and immunofluorescence imaging methods allow such segmentation to a certain extent, but resulting segmentation masks are only valid for a limited set of target proteins due to morphology changes in consecutive tissue slices. For multi-protein analysis, an image registration process is therefore commonly needed to align individual cells of consecutive images to the mask.

Multiplexed mass cytometry eludes this effort by simultaneously scanning multiple proteins on the same slice. We introduce a new single cell segmentation method on highly multiplexed images which exploits the highly registered multidimensional cell boundary information of multiplexed immunohistochemical mass cytometry imaging. The joint information of the cell-cell junction membrane proteins β-Catenin, HER2 and Cytokeratin 8/18 as well as the histone protein H3 serve as input for the watershed.

For quantitative validation without manually segmented gold-standard, we define a new segmentation score which considers the size of cells, the number of nuclei per cell and the overlap of segmentation with membrane and nuclei signal. The score allows the comparison of different segmentation masks. Our method is tested on a human breast cancer dataset with FFPE tissue specimens.

**Results and Discussion:** Single cell segmentation on multiplexed mass cytometry images is very accurate due to highly registered image information of multiple membrane protein channels. The weighted combination of 0.63*β-Catenin 0.25*HER2 and 0.12*Cytokeratin 8/18 gave the best segmentation, demonstrating different contribution of individual channels. Segmentation masks can be used for statistical analysis of dozens of targeted proteins.

**Conclusion:** We propose a new method of cell segmentation exploiting highly registered multidimensional membrane information on a new kind of immunohistochemical imaging. We have shown that individual membrane protein channels contribute to the segmentation with different intensity: a weighted combination yields the best segmentation. Such relations are difficult to show on conventional imaging techniques where a prior registration of multiple channel images from consecutive slices is necessary. Further, we provide a cell segmentation score respecting the expectations of a valuable segmentation with which we can compare different segmentation masks.
B2: IT In Pathology

Preliminary results from a crowdsourcing experiment in immunohistochemistry

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ITALY

Background: Crowdsourcing, i.e., the outsourcing of tasks typically performed by a few experts to a large crowd as an open call, has been shown to be reasonably effective in many cases, like Wikipedia, the Chess match of Kasparov against the world in 1999, and several others. The aim of the present paper is to describe the setup of an experimentation of crowdsourcing techniques applied to the quantification of immunohistochemistry.

Methods: Fourteen Images from MIB1-stained breast specimens were first manually counted by a pathologist, then submitted to a crowdsourcing platform through a specifically developed application. 10 positivity evaluations for each image have been collected and summarized using their median. The positivity values have been then compared to the gold standard provided by the pathologist by means of Spearman correlation.

Results: Contributors were in total 28, and evaluated 4.64 images each on average. Spearman correlation between gold and crowdsourced positivity percentages is 0.946 (p<0.001).

Conclusions: Aim of the experiment was to understand how to use crowdsourcing for an image analysis task that is currently time-consuming when done by human experts. Crowdsourced work can be used in various ways, in particular statistically aggregating data to reduce identification errors. However, in this preliminary experimentation we just considered the most basic indicator, that is the median positivity percentage, which provided overall good results. This method might be more aimed to research than routine: when a large number of images are in need of ad-hoc evaluation, crowdsourcing may represent a quick answer to the need.

A model based interface terminology for generic observations in Anatomic Pathology Structured Reports

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Introduction: Current terminology systems for structured reporting in pathology are more or less focussed on tumor pathology. They have not been compiled in a systematic approach, therefore they gather terms of very different granularity. Generic models for terminology development could help in establishing reference terminologies for all fields of anatomic pathology. The core principle of those models is the ontological structure of native speaking terminology. Each term can be seen in a few generic aspects of observation: The target of observation, attributes of that target with a set of possible qualifiers, a locator and a problem identifier.

By analyzing the PathLex interface a generic terminology model will be derived.

Material and Methods: For each element template of PathLex its possible generic nature and its value set was analyzed, looking for the uniqueness or multiplicity of the values in the value sets. The generic terms were mapped to SNOMED-CT terms using “ArtDecor”.

Results: The 488 PathLex element templates for Anatomic Pathology (AP) observations can be reduced to 53 generic templates, leaving out only 17 templates very specific for organ and/or disease. Among those 53 templates 28 are describing UICC-TNM staging, ICD-O-classification, and grading. Further 15 templates describe the results from marker investigations. Almost all of those generic terms could be mapped to SNOMED CT.

All of the generic elements have their “organ specific” counterparts by assigning them to one of 20 organs and invasive or noninvasive cancer, respectively. Studying the structure of generic and specific terms it becomes obvious that any AP observation
- occurs always in a context
- consists of four basic elements (target of observation, Attribute of observation, additional qualifiers and a value set).

Based on the generic elements of PathLex 22 generic expressions of general pathology, 9
generic targets and 12 qualifiers as well as 4 relationships were defined.

**Discussion:** If a machine-readable terminology is aimed to preserve all the information of native speaking, then two principal solutions exist:

- systematic consideration of all the aspects mentioned above in each single term
- focusing on the generic elements of terms and combining this with the structure of communication, reflecting the non-obvious elements of the terminology.

The fastest way for establishing an interface terminology is the first approach, which lists all of the terms needed for e.g. a checklist in a comprehensive manner (precoordination).

However, if the list of terms and problems increases, or new requirements have to be met, considerable difficulties may arise in keeping the terminology consistent and complete.

The second, postcoordination approach offers some advantages. It does not have limitations in the organ- or disease specificity, and it keeps the number of terms limited, making them more easily to survey.

The generic approach does not only influence the terminology itself, but depends also on the consideration of context information. The decisive criterion for generic nature of a term is its value set. If ever possible, generic terms should be used.

**Crowdsourcing Mitosis count: an experiment on the MITOS dataset**

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*ITALY*

**Introduction:** Crowdsourcing, i.e., the outsourcing of tasks typically performed by a few experts to a large crowd as an open call, has been shown to be reasonably effective in many cases, like Wikipedia. Several crowdsourcing have also appeared on the Web: they allow requesters to post the tasks they want to crowdsource and workers to perform those tasks for a small reward.

One classical crowdsourcing topic is image recognition, in the form of image tagging and moderation for usage in image databases, forums, etc. Aim of the present extended abstract is to describe the setup of an experimentation of crowdsourcing techniques applied to mitosis count and tested on the MITOS dataset, already used for an international image analysis contest (1).

**Material and methods:** A web-based application has been developed for hosting images, on which users could evidentiate mitoses by clicking on them. Images were then proposed as task in a crowdsourcing platform, allowing up to ten workers to execute each. After a first test run with a free number of clicks (ended with too much noise), we decided to provide each worker with 20 clicks per image to identify the most likely candidates. In this first experiment, 7 images (2084x2084 pixels) were proposed to workers, that included 42 mitoses in total.

Worker selections have been clustered with the DBSCAN algorithm on the R software, and compared against the MITOS ground truth using Euclidean metrics. Finally, the number of true and false positives were calculated.

**Results and discussion:** The total running time for the experiment was 145 minutes, and involved 30 workers, which analyzed 2.3 images each on average (1-7). After clustering (with parameters: 25 as reachability distance, 5 as minimum number of clicks), the total number of mitoses identified was 62, of which 27 true positives (64% of true mitoses) and 35 false positives.

**Conclusion:** Aim of the experiment was to understand how to use crowdsourcing for an image analysis task that is currently time-consuming when done by humans, and somewhat difficult, although feasible, if done by software, as documented by the MITOS contest. Crowdsourced work can be used in various ways, in particular relying on the crowd to reduce identification errors (e.g., by considering the most selected cells, or the cells selected above a threshold, etc). In this preliminary experiment we set up a method that might be adequate for a difficult task like mitosis identification, but some further analysis is needed to better exploit the crowd wisdom.

**Field testing of structured reporting using a PACS- based patient data management system.**

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*CROATIA*

**Introduction:** The role and position of pathology in the healthcare system changes rapidly. The pathologist has to take over the role of an integrator of diagnostic and therapeutic actions abandoning the classical bystander role. Structured reporting is one of features positioning data produced by the pathologist into the focus of clinical research. The goal of a structured report is to capture all key elements from the report e.g.
clinical, macro and microscopic, and enable their integration into the Clinical Path of a given patient but also facilitate meta-analysis and integration of large cohorts of patients for clinical studies. Croatian Society of Pathology formulated and published 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 supposed to be checked on a preformatted organ specific hierarchically organized. We adapted a specialized Pathology information system (PIS) and pathology PACS (being the part of HIS) in order to comply with these demands.

**Objective:** Testing of a PIS/PACS-based workflow and data management system including defining, organizing and editing structured pathology reports and integrating them into full text reports in order to facilitate the exchange of structured pathology reports within the healthcare system. Testing the accessibility and usefulness of these data for other non-pathologist colleagues.

**Methods:** The system (ISSA, VAMS Tech, Zagreb, Croatia) was tested on checklists for different organ systems (GI tract, breast, gynecology, lung, bone and soft tissue pathology) with variable extent of checklist data. From checklists free text reports were generated.

**Results:** Introduction of structured reporting is easily possible using a PACS system equipped with hierarchically structured checklists based on professional guidelines. A readable full-text report can be constructed from the checklist. For the general pathologist working a broad range of different diagnostic areas the checklist presents a most valuable tool for report standardization and quality assurance. The produced data, if they are integrated in the HIS can readily be used by all accredited doctors.

**Conclusion:** Obviously a step further toward integration of all patient data has to be taken. A question not to be neglected in the future is whether the plethora of data gathered by pathologists and readily sheared in a most organized form with other colleagues will lead to less pathologists being included into scientific publications organized by clinical leading authors bringing the pathologists again on the margin of events.

**Virtual Microscopy in Modern Tissue-Biobanks - the ZeBanC example**

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**Introduction:** Biobanks are key resources for healthcare to identify new potential markers that can be used in patient diagnosis and complement the targeted personalized drug treatments. There is an ongoing development in the direction of the establishment of central biobanks with respect to improvements in standardization and cooperation with the aim to establish relevant case collections. The ZeBanC is the central biomaterial bank of the Charité in Berlin. Virtual microscopy (VM) offers huge potential in tissue biobanks. Due to an expansion to aspects of quality control and documentation as well as to image analysis, ZeBanC – related projects dealing with tissue specimens can immediately benefit from virtual microscopy enabled biobanking.

**Materials and Methods:** The introduction of VM in the ZeBanC context offers several advantages and opportunities:
- Immediate electronic access to WSI by staff and customers
- Computer aided navigation (e.g. parallel visualization of biomarkers)
- Continuous and SOP based quality control (e.g. amount of tumor in tissue)
- Completion of missing attributes using image analysis
  - Seamless integration of TMAs
  - Realization of “virtual studies” based on series of virtual slides as additional option

We developed a specialized software application for handling of WSIs in the context of the ZeBanC. This tool offers probe identification via barcode or OCR, validates completeness of tissue digitalization as well as image sharpness using automatic image analysis. Subsequently sections of the same block can be scheduled for automatic registration.

Integrated image analysis will be a key feature of VM in biobanking, in addition to simpler handling as well as access and quality control. Several measurements are important for any kind of tumor probes in the biobank. This includes the determination of the tumor area, the characterization of inflammation in the tumor or the detection of the different tissue types. Putting these parts together we can formulate search requests or questions of the form

a) “Search for paraffin blocks of breast carcinomas with tumor area larger than 1 mm², HER2 score 3+, with a follow-up of 5 years or longer, where blood is available.”
b) “What is the ratio between the numbers of tumor cells and lymphocytes in Luminal A and in Luminal B breast carcinomas?”

**Results and Discussion:** The integration of VM and biobanking is at the end of the day the establishment of interfaces between biobank IT system, pathology IT system, virtual microscopy system and image analysis as well as the development of software tools offering additional functionality by combining the potentials of the systems. Within the ZeBanC we developed several such tools which allow sophisticated functionality as well as virtual studies on virtual slides against the background of real samples.

**Conclusion:** Virtual microscopy is a technology which will influence the development of future biobanking parallel to technologies in the context of next generation sequencing.
Exploring the Spatial Dimension of Estrogen and Progesterone Signaling: Detection of Nuclear Labeling in Lobular Epithelial Cells in Normal Mammary Glands Adjacent to Breast Cancer

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Introduction: Aim of this work was the analysis of spatial aspects of estrogen receptor (ER) and progesterone receptor (PR) nuclear positivity in lobules of the mammary gland adjacent to breast cancer. Assessment of nuclear markers in specific cells and tissue areas requires image analysis at high resolution, exceeding reasonable processing time in Whole Slide Imaging (WSI). To limit the analysis to relevant areas of interest, a common workaround in digital pathology is manual interaction of pathologists who annotate relevant regions. Herein, we propose automated detection of lobular structures adjacent to breast cancer, limiting human interaction largely to subsequent quality control.

Material and methods: A training set (n=6) and a test set (n=12) of hormone receptor negative ductal invasive breast cancer were selected and 2-4 µm thick paraffin sections were stained using automated ER and PR detection (Ventana Benchmark Ultra). Slide scanning was performed using AperioScanScope at 20x magnification, and saved in svs format using JPEG2000 compression. The image analysis software Definiens Developer was used for image analysis. Whole slide images were downsampled to 10% of original resolution and classified into tissue and background. Images were segmented at five levels of granularity, using Definiens’ multiresolution segmentation. Initial lobule candidates were classified based on textural, geometric and relational features. In a second step, candidates were evaluated on each level and if necessary reclassified based on properties of sub-segments at the next finer level. Inside the lobules, positive and negative nuclei were detected on the original resolution, and the nuclear positivity was calculated. Tumor was classified based on growing seed segments, and lobules were divided into three categories based on their distance to the tumor (adjacent: <0.5mm; intermediate: 0.5-2mm; distant: >2mm).

Results and discussion: We identified the mean standard deviation to neighbor pixels within a segment as a critical textural feature for lobule detection. This relatively simple feature was found to be a strong discriminator between lobules and the surrounding tissue, most likely reflecting the morphology of tubular glandular structures. As geometric features, we established the size of the lobule candidates and a minimum roundness criterion. The most important relational features were coupled to textural features: border contrast and mean difference, both calculated for the mean standard deviation, were used to evaluate the relations to neighbor segments. Average processing time was 45 min per case on a PC. After rule set optimization in the training set, we found acceptable automated lobule detection quality (40-80% true positives) in 4/12 (33%) of cases. ER nuclear staining was consistently lower in lobules in close proximity to the tumor bulk than in distant lobular structures. In 8 cases, lobule detection was insufficient due to brisk inflammatory infiltrates disrupting the lobular structures.

Conclusion: Our approach enables assessment of ER/PR signaling in mammary gland tissue in the spatial context of breast cancer. Current limitations to be addressed by further rule set development include brisk inflammatory infiltration disrupting the normal morphological structure of the tubular gland epithelium.

Compartment based automated IHC cell and tissue analysis

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Introduction: The immunohistochemical (IHC) stains are widely used as diagnostic procedures in pathology. This method can reveal the target proteins of malignant lesions which could have predictive or prognostic clinical roles. In this study we aimed to develop a new image segmentation algorithm special for cell based IHC histological analysis in cancer tissue. This algorithm was optimized to scanned whole slide quantification so this could be an effective device for digital pathology.

Findings: First we defined a digital slide collection which could be a basis of the algorithm development process. This slide set represented a
wide range of IHC stainings with different quality. The selected antibodies stained target proteins with different localizations (nuclei, cytoplasm membrane markers). We established that the chromogen and fluorescent signals presented into different cell compartments resulted typical cases where the morphological representation of stained cells were characteristic. We developed our own image segmentation algorithm which covered each of defined morphological cases.

The algorithm builds up from cell compartment specific modules which have hierarchical relationship to each other. The detection and the classification algorithm run on the cell nuclei, cytoplasm and membrane level parallel and extract characteristic features of these objects.

**Conclusion:** Our preliminary results showed that the applied image segmentation procedure was suitable for IHC stained tissue quantification. The available input slide set seemed to be sufficient to get the basis of the development process. Finally, we presented a software solution which could be a helpful device for pathological diagnostics, but before the introduction of in vitro diagnostic processes further investigations and validation studies are needed.

**Automatic segmentation, classification and alignment of consecutive sections for block centric navigation**

_N. Zerbe, K. Schlüns, B. Lindequist, P. Hufnagl_  
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**Introduction:** Pathology evaluates topological and morphological and immune-histochemical changes in histological structures based on tissue sections. One section offers only limited information but most diagnostic decisions require multiple sections, stains or even both. Not only conventional but also digital pathology proves low efficiency in block-based slide handling and colocalization of regions of interest through multiple sections of the same block. Biopsies and surgical specimens have different configurations of tissue. In general multiple biopsy sections are mounted on one glass slide. Here they may contain several tissue particles. In contrast only one or two sections of surgical specimen are mounted to one slide containing one tissue particle only. We introduce a method for an automatic analysis of serial sections of both specimen types in a routine environment to extract required information for subsequent block centric navigation.

**Material and Methods:** Due to multiple applications within the field of computer assisted navigation such as parallel viewing, parallel annotating, virtual staining, virtual stacks and 3D reconstruction, it is conducive to provide extracted correspondence information independent. Thus, we separated analysis into modules to combine them as requires and to divide computation into offline and online parts. This enables preprocessing and extraction of correspondences offline, prior to the diagnostic process. In parallel this provides flexibility to use gathered information online appropriately to the actual use-case.

Subsequently to an initial contour based segmentation of tissue particles in a low resolution analysis is divided into two main parts, a) recognition of sections and classification of tissue particles and b) correspondence analysis.

- a) Sections are not explicitly represented within slides. For that reason we identify them indirectly. Based on all identified tissue particles an alpha shape and its medial axis are calculated to approximate to serialization curve of sections. This approximation is used to reconstruct sections using a weighted line-scan algorithm. In a next step particles are classified using shape and size based features to identify corresponding particles and reconstruct serialization.

- b) We calculate coarse alignment of particles described by a rigid transformation. For this purpose we implemented an iterative closest point (ICP) method based on inner and outer contours of corresponding particles. Further refinement is achieved by a high resolution piecewise correspondence analysis using the SIFT method.

**Results and Discussion:** The introduced method was applied to routine cases to extract high resolution point based correspondences for navigation. The grade of automation as well as the quality of results is directly dependent to the presence of artifacts and tissue distortions caused by tissue preparaion. Moreover, registration of certain stains show weaknesses due to strong visual differences of tissue texture. First experiments suggest an object based refinement to solve this issue.

**Conclusion:** Computer assisted navigation is emerging. Recently, full automatic analysis of WSI to extract correspondences for multiple applications within the navigation domain is demonstrated for routine material. Future prospects are a seamless integration of analysis into digitalization and slide providing process. Together with appropriate user-interfaces to operate within the different use cases block
centric navigation offers a method to improve not only reproducibility but also efficiency.

**Automated interpretation system of microscopy image for ER/PR assessment in breast cancer from a fuzzy intelligence system.**

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**SPAIN**

**Introduction:** Breast cancer is the most frequent cancer among women and its first cause of death in the world. The Estrogen Receptor (ER) and Progesterone Receptor (PR) status have a great predictive value in the benefits of hormonal therapy and a prognostic value in the evolution of the disease. The evaluation of immunohistochemistry stained tissues is usually carried out by the pathologist with a conventional microscope. The introduction of digital microscopy at Pathology laboratories shows maturation of the technology and the application of computer vision techniques to help the pathologist become possible.

**Material and Methods:** A new image processing system based on fuzzy intelligence is proposed. This system performs automated interpretation of ER/PR status for images captured from bright field microscope autonomously without the intervention of the pathologist.

The implementation of the image processing system is written in C and Java languages using the free OpenCV 2.1 and ImageJ 1.44 vision libraries. Comparisons among automated interpretation system of microscopy image (AISMI), the free web available ImmunoRatio, original assessment in laboratory (P2) and pathologist (P1 - Gold Standard) was performed.

**Results And Discussions:** The results showed a good correlation between our algorithm and ImmunoRatio (0.971), and also both of them with manual assessment of images (AISMI - P1 0.863; ImmunoRatio-P1 0.796); the greatest differences between P1 and automatic assessments were found in images with high proportions of stromal nuclei over glandular nuclei and those with a low percentage of stained nuclei; Furthermore, the correlation coefficient between pathologists assessments (P2-P1) was 0.35, highest differences becomes in images with a low percentage of stained nuclei area.

**Conclusion:** The assessment differences on stained tissues using methods based on robot vision and pathologist are similar to those found between pathologists. Image processing times are very low for the proposed system. The proposed system can be used as an effective diagnostic support allowing a quick analysis of several microscopic images with no pathologist interaction and using common lab equipment.

**Texture-based segmentation and lesion’s graduation of placenta structures in spontaneous miscarriage**

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**POLAND**

**Introduction:** The paper presents method of segmentation and lesion’s graduation of placenta structures in spontaneous miscarriage. We propose texture method applicable to heterogenous microscopic images representing histological slides of placenta. In solution we employ modified Unser local texture descriptions of placenta image subregions that are grouped around succeeding pixels of image.

**Material and methods:** Twenty histological slides come from archives of the Department of Pathology, Military Institute of Medicine, Warsaw, Poland. They were stained with hematoxylin and eosin, and images were acquired at 400x. Our task is to build computerized system able to recognise different classes of objects.

The proposed image analysis scheme is organized in the following steps: description of local image properties using textural features, classification of pixels applying a classifier, grouping pixels into compact subregions and correction of borders between subregions using region growing method. Procedure of texture analysis is organized in few steps. First, we create sum and difference images for three RGB channels on the basis of original image and image translated by 3 pixels. Next, we select the set of neighborhood region masks for each pixel location to find the texture descriptors. Calculated features can be used as input attributes to classifiers - support vector machine and random forest. As a result of such classification each pixel is associated with a class. Pixels form closed regions representing mixed texture properties. Some corrections at borders between regions should be done using region growing method. The developed algorithm allows to assess which features are the most crucial in class recognition. We employed relieff algorithm ranking features
according to how well their values distinguish between instances near each other.

**Results:** For each RGB component we generated eight modified Unser textural features. In this way each pixel is characterized by 24-dimentional vector forming input signals to classifier. The classifier was learnt six classes of pixels representing: villous mesenchyme, fibrosis, trophoblast, hemorrhage, trophoblast proliferation or bearing partitions and background. Interior regions of the villi with a number of loosely distributed mesenchymal stromal cells were correctly identified with 94.7% of accuracy. The background area has been recognized with 97.2% of accuracy, the fibrosis with 91.7% and the trophoblast with 92.9%.

The descriptors of pixels were applied simultaneously to make graduation of the specific image regions. We used them to identify swelling degree of mezyncheme in villi. Three classes were recognized: no swollen villi, medium and large swollen villi. The classes of villis were assessed by experts and recognized by system using random forest classifier. The average recognition accuracy of 3 classes was equal 96.1%.

**Conclusions:** We developed new approach based on generation of textural features describing local properties of images and classification of pixels. The proposed method was used in recognition of internal structures of placenta tissues in spontaneous miscarriage. Numerical results have shown satisfactory accuracy of structure recognition.

**Prediction of Cardiovascular Death by Cardiac and Aortic Morphometry – a Preliminary Autopsy Study**

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**ROMANIA**

**Introduction:** The aim of the study is to make a preliminary assessment of the neural network usefulness in discriminating between cardiovascular and non cardiovascular causes of death by analysing of some cardiac and vascular morphometric parameters.

**Material and Methods:** Autopsies were made in 81 cases, 55 out of which having a non-cardiac cause of death and the rest of 26 having a cardiac cause of death. There were assessed 5 cardiac parameters, measured on the third transverse section starting from the apex (the thickness of left ventricle anterior, lateral and posterior walls, septum and right ventricle wall) and 5 vascular parameters (the diameters of the pulmonary artery and of the ring, ascendant, thoracic and abdominal aortic segments) for each case. The collected values were used together with subject’s sex and age to train a two-layer feed-forward neural network, designed as follows: 70% of the data used for training, 15% used for validation and 15% used for testing.

**Results:** The network output had considerable precision with about 80% correct prediction rates. Thus, the model could be considered as highly relevant taking into consideration the reduced amount of the information it used.

More than that approximately the same measures can be made with the use of echocardiographs. These measures can be made while the subject is still alive (in these case some correlations must be made between artery diameters caused by normal blood pressure) and/or after it’s death.

**Conclusion:** Taking into consideration that the above mentioned measurements could be determined in vivo, using the echocardiograph, the obtained results showed that the developed model has a great practical potential in signaling the risk of death by a cardiovascular cause.
Towards better digital pathology workflows: Programming libraries for high-speed sharpness assessment of Whole Slide Images
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FRANCE

Introduction: Since microscopic slides can now be automatically digitized and integrated in the clinical workflow, quality assessment of these Whole Slide Images (WSI) has become a crucial issue. Our patented method consists in a no-reference quality assessment of WSI that has been thoroughly tested in the last four years and which is currently being implemented in our university-hospital. It is also part of the FlexMIm project, which aims to improve the global workflow of digital pathology. This project, funded by an R&D grant of the French government, also involves universities Paris 6 and Paris 7, LIAFA, IPAL and LIP6 laboratories, industrial partners Orange Healthcare, Pertimm and TRIBVN and 27 anatomo-pathological centers in the Paris region. For these projects, we have developed two programming libraries, in Java and Python, which can be integrated in various types of WSI and image handling applications.

Material and Methods: The development has been carried out on a MacBook Pro (i7, 2012) and tests were carried out in University Paris Diderot (bi-Xeon 2.7GHz, 2012). The libraries implementing the blur assessment method have been developed in Java, Python, PHP5 and MySQL5. For web usage, JavaScript, Ajax, JSON and Sockets were also used, as well as the Google Maps API, as demonstrated in NYU’s virtual microscope (NYUVIM). Aperio SVS files were converted into the Google Maps format using VIPS and Openslide libraries.

Results and Discussion: The WSI sharpness analysis Java library was designed as a Service Provider Interface (SPI): an Application Programming Interface extendable by third parties. We designed 4 sharpness assessment programs based on our Java multithreaded library. One using regular images or array of images, with text-only results. One using Hamamatsu NDPI WSI, and returning global results for the slide sharpness at each magnification, as well as a sharpness map of the WSI summarizing the results with colors relative to the sharpness detected. One similar program using the Google Maps format, and one web application using the Google Maps format to be viewed with the NYUVM. The sharpness analysis of the tiles are computed and sent concurrently and faster than the images are displayed, thereby in real-time.
Tests were made on 5000 single images, 200 NDPI WSI, 100 Aperio SVS WSI converted to the Google Maps format.

Conclusions: These sharpness assessment libraries are currently being implemented in our hospital and in the FlexMIm project and new results should be provided in the end of 2014. Applications based on our libraries can be used upstream, as calibration and quality control tool for the WSI acquisition systems, or as tools to reacquire tiles while the WSI is being scanned. They can also be used downstream to reacquire the complete slides that are below the quality threshold for surgical pathology analysis. WSI may also be displayed in a smarter way by sending and displaying the regions of highest quality before other regions. Such quality assessment scores could be integrated as WSI’s metadata shared in clinical, research or teaching contexts, for a more efficient medical informatics workflow.

Digistain: A Digital Staining Instrument for Histopathology
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UNITED KINGDOM

Introduction: To report a new technique for providing a quantitative measure of breast tumour grade in breast core biopsies.

Method: Digistain images are derived from the relative concentrations of the amide (cytoplasmic) and phosphate (nuclear) moieties present in tumour cells. Reflecting the Nuclear/Cytoplasmic ratio in tumour cells, they allow these respective concentrations to be evaluated as a measure of malignancy. A suitably calibrated combination of these concentrations is used to generate false colour computer images that reproduce not only tissue morphology, but also accurate and quantitative maps of chemical composition throughout the tissue section. Unlike other digital pathology tools employed to assist diagnosis, Digistain uses a unique optical signature to
analyse the chemical make-up of a biopsy quantitatively, using unstained sections. The technique is unaffected by the subjectivity of grading, particularly grade 2 and intermediate cases. Within minutes of loading a slide it yields a reproducible and numerical grading score that helps physicians and patients decide on the most effective treatment plan.

**Results:** 20 breast cancer core biopsies were classified according to their respective grades. The Digistain score of each group was found to correlate strongly across the grades, thus validating the use of this index as a suitable indicator of malignancy.

**Conclusion:** We believe the new Digistain approach provides for the first time a cost effective and quantitative measure of tumour grade. This can be developed to deliver an effective assessment of prognosis and recurrence risk beyond traditional qualitative measures based on H & E staining protocols.

**iPathology cockpit diagnostic station: validation according to College of American Pathologists Pathology and Laboratory Quality Center recommendation at the Hospital Trust and University of Verona.**

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**ITALY**

**Background:** Validation of digital whole slide images is crucial to ensure that diagnostic performance is at least equivalent to that of glass slides and light microscopy. The College of American Pathologists Pathology and Laboratory Quality Center recently developed recommendations for internal digital pathology system validation. Following these guidelines we sought to validate the performance of a digital approach for routine diagnosis by using an iPad and digital control widescreen-assisted workstation through a pilot study.

**Materials and Methods:** From January 2014, 61 histopathological slides were scanned by ScanScope Digital Slides Scanner (Aperio, Vista, CA). Two independent pathologists performed diagnosis on virtual slides in front of a widescreen by using two computer devices (ImageScope viewing software) located to different Health Institutions (AOU1 Verona) connected by local network and a remote image server using an iPad tablet (Aperio, Vista, CA), after uploading the Citrix receiver for iPad. Quality indicators related to image characters and work-flow of the e-health cockpit enterprise system were scored based on subjective (high vs poor) perception. The images were re-evaluated two weeks apart.

**Results:** The whole glass slides encountered 10 liver (hepatocarcinoma), 10 renal (carcinoma), 10 gastric (carcinoma) and 10 prostate biopsies (adenocarcinoma), 5 excisional skin biopsies (melanoma), 5 lymph-nodes (lymphoma), 6 immuno- and 5 special stains were available for intra- and internet remote viewing. Scan times averaged two minutes and 54 seconds per slide (standard deviation 2 minutes 34 seconds). Reliance on glass slide, image quality (resolution and color fidelity), slide navigation time, simultaneous viewers in geographically remote locations were considered of high performance score. Side by side comparisons between diagnosis performed on tissue glass slides versus widescreen were excellent showing an almost perfect concordance (0.81, kappa index).

**Conclusion:** We validated our institutional digital pathology system for routine diagnostic facing with whole slide images in a cockpit enterprise digital system or iPad tablet. Computer widescreens are better for diagnosing scanned glass slide that iPad. For urgent requests, iPad may be used. Legal aspects have to be soon faced with to permit the clinical use of this technology in a manner that does not compromise patient care.

**Mitotic score in breast cancer: digital counting versus usual microscopic**

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**FRANCE**

**Introduction:** mitotic recognition and counting is mandatory for grade, classification, and prognosis of breast carcinomas. The count usually stands as a decisive point for treatment. Counting mitoses is done by screening routine H&E stained slides.

**Material and Methods:** 16 cases of invasive ductal breast carcinoma were reviewed on routine H&E based on their Elston Ellis modified SBR grade: 9 of grade III, 4 of grade II and 3 of grade I. They were then comparatively screened on whole slides images (virtual slides/digital slides) by two different pathologists at 40 magnification using a
Hamamatsu scanner (resolution: 0.24 micron/pixel) and visualized by CaloPix software developed by TRIBVN in order to determine inter observer and intra observer variations. Mitotic score was made according to the WHO recommendation that is dependent on microscopic field area.

Results and discussion: the mitotic count, performed by both pathologists, on numerical images leads to a better rate than inter observer agreement on microscopic slides (Cohen’s Kappa 0.73 for virtual slide, 0.60 for microscopic slide and p value < 0.001). However, the agreement between the 2 techniques on mitosis count was moderate. This probably results from the fact that it overestimates the number of mitoses counting on microscope slide. This did not change the final Elston Ellis score with a strong agreement between the 2 techniques (Cohen’s Kappa of 0.90).

The main advantages of mitosis count on whole slide image are reproducibility, traceability and thus quality. A review or check of the score can be standardized if the score is at the cutting edge with an impact on the global grade. The validation of digital pathology as a routine practice should include validation and comparing of the standards ancillary and new procedure. This implies the digital network of the department includes such options for quality, extended to Tele pathology practice. It also implies that shape recognition of mitosis on virtual slides should be validated according to a standardized digital multi observer procedure.

Conclusion: telepathology will eventually permit discussing the diagnosis between pathologists with recommendations to give the mitotic count on virtual slide and microscope slide on the final report.

Multiple immunohistochemistry markers by digital image analysis reveal complex interdependencies but do not provide prognostic value in multivariate analysis models

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Introduction: In this study, we aimed to test feasibility of integrated indices derived from multiple IHC markers measured by DIA tools to determine interdependencies and clinical behaviour of the PCa.

Material and Methods: Tissue microarrays (TMA) were constructed from PCa tissue (3 cores of 1 mm per patient) obtained by radical prostatectomy from 192 male patients without adjuvant therapy. TMA were stained with six (IHC6) monoclonal antibodies against ERG, p504s, androgen receptor (AR), Ki67, p53, p16\(^{INK4a}\) (p16) antigenes. DIA was performed with Aperio Nuclear V9 or Aperio Cytoplasmic algorithms (for 659 cores). Visual evaluation (VE) of the images was performed by a qualified pathologist (286 tumor cores). For statistical analysis other data sets were used: clinical variables - patient age, prostate weight (g), and ranked pathological data - Gleason score (6 to 9), pTpn (from T1aN0 to T3cN1), tumour volume (visual percent of PCa in prostate slides).

Results and Discussion: Inter-method agreement for DIA with VE by single linear regression method was good for ERG and Ki-67 (R\(^2\) = 0.72 and 0.65, respectively) and fair for p16, p53 (R\(^2\) = 0.37 and 0.39, respectively) in each TMA core (n=286). DIA results were used for further calculations. FA in 166 patients with full set of data revealed strong positive loadings in factor 1 pattern for Gleason, pTNM, and tumour volume (Vt) - 0.67, 0.78, 0.75, respectively. The factor 1, in essence, corresponded to the Epstein criteria of pathologically insignificant prostate cancer and was therefore named “Epstein” factor. Whereas, factor 2 with Ki67, AR and ERG positive loadings of 0.86, 0.82 and 0.49, respectively, was consistent with the findings of previous studies, disclosing interdependences for aggressiveness and proliferation, it was named “AR-ERG-associated proliferation”. Factor 3 with the loadings of p16 (0.85), ERG (0.59) and p53 (0.53) was suggestive of genetic interdependence of “cell cycle and proliferation control”. Factor 4 for prostate weight (0.79) and patient’s age 0.68)
could reflect age-dependent increase of prostate weight (“Age” factor). Factor 5 for p504s (0.85), p53 (0.58) was difficult to interpret.

There were 27 PSA biochemical recurrence (BCR) events (21% in 131 patients with follow-up data available. Kaplan-Meier survival estimates revealed pT 1a-2c (p=0.0001), Gleason score 3+3, 3+4 (p=0.001) and Vt <16% (p=0.003) to have a better BCR-free survival. In multiple Cox regression, significance of odds ratio at proportional hazards for post-operative BCR (1.93) was p=0.0014 for the “Epstein” factor. In multiple Cox regression; other factor scores as independent variables as well as single IHC by DIA or VE had no significant impact on the BCR.

**Conclusion:** Our study shows that multivariate analysis of multiple IHC expression by DIA in the context of conventional clinical and pathology variables of PCa extracts complex but meaningful interdependencies in the data set and highlights informative value of single parameters. However, only the “Epstein” factor score was an independent prognostic indicator of post-operative BCR in this data set.
B5: Quality-2: Quality Control

Digital immunohistochemistry wizard: image analysis-assisted stereology tool to produce reference data set for calibration and quality control
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Background: Digital image analysis (DIA) enables better reproducibility of immunohistochemistry (IHC) studies. Nevertheless, accuracy of the DIA methods needs to be ensured, demanding production of reference data sets. We have reported on methodology to calibrate DIA for Ki67 IHC in breast cancer tissue based on reference data obtained by stereology grid count. To produce the reference data more efficiently, we propose digital IHC wizard generating initial cell marks to be verified by experts.

Methods: Digital images of proliferation marker Ki67 IHC from 158 patients (one tissue microarray spot per patient) with an invasive ductal carcinoma of the breast were used. Manual data (mD) were obtained by marking Ki67-positive and negative tumour cells, using a stereological method for 2D object enumeration. DIA was used as an initial step in stereology grid count to generate the digital data (dD) marks by Aperio Genie and Nuclear algorithms. The dD were collected into XML files from the DIA markup images and overlaid on the original spots along with the stereology grid. The expert correction of the dD marks resulted in corrected data (cD). The percentages of Ki67 positive tumour cells per spot in the mD, dD, and cD sets were compared by single linear regression analysis. Efficiency of cD production was estimated based on manual editing effort.

Results: The percentage of Ki67-positive tumor cells was in very good agreement in the mD, dD, and cD sets: regression of cD from dD (R²=0.92) reflects the impact of the expert editing the dD as well as accuracy of the DIA used; regression of the cD from the mD (R²=0.94) represents the consistency of the DIA-assisted ground truth (cD) with the manual procedure. Nevertheless, the accuracy of detection of individual tumour cells was much lower: in average, 18 and 219 marks per spot were edited due to the Genie and Nuclear algorithm errors, respectively. The DIA-assisted cD production in our experiment saved approximately 2/3 of manual marking.

Conclusions: Digital IHC wizard enabled DIA-assisted stereology to produce reference data in a consistent and efficient way. It can provide quality control measure for appraising accuracy of the DIA steps.

Accuracy of an Automated Vessel Counting Algorithm in Four Different Tumor Types
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BELGIUM

Introduction: Currently, considerable attention is being paid to developing predictive biomarkers for anti-angiogenic therapies, but none exist to date. Important causes are the complex action mechanism of the therapy, study size, tumor heterogeneity, and the lack of standardized methodology. In an attempt to standardize microvessel density measurements, we compared a commercial image analysis platform (Definiens Architect) with our manual method.

The most popular approach to measure angiogenesis is to count the smallest vessels (microvessels) in a tissue section of tumor.¹ These vessels are visible at high magnification (200x – 400x). We compared the results of our manual scoring method with the results produced by a commercial image analysis platform (Definiens Architect). Automated image analysis eliminates human subjectivity and enhances reproducibility.² High throughput is evident with these systems. The major limitation of image analysis relates to its accuracy, which needs to be tested and cross-validated.

Material and Methods: From the archives of HistoGeneX (Antwerp, Belgium), 82 tissue slides were selected based on previous vessel counts. Based upon the topological heterogeneity of the vessels in the section, three groups were selected: low, medium and high. All slides were stained with the same protocol for CD31, a pan-endothelial marker used for vessel detection. A pathological report for every slide was available and used for the regional selection of tumor tissue. The manual method consisted of random and systematic sampling using stereological techniques for the selection of regions of interest.³ Fifteen regions per tumor were selected. In total, 1230 regions were overlaid with a rectangular grid
and analyzed for the number of vessels by two observers.

The automatic method consisted of the Blood Vessel Analysis algorithm built into Definiens Architect XD 2 (Definiens AG, Munich, Germany). The exact same area under the grid was used as for manual analysis. Four types of cancer tissue were selected: colorectal cancer (CRC), glioblastoma multiforme (GBM), ovarian cancer (OC) and renal-cell carcinoma (RCC). The settings for each tumor type were optimized, with the following CRC settings: IHC Marker = Membrane, Magnification = 20x, IHC Threshold = 0.20, Min Stain Area = 175, Gap to close = 8. Classification results (Figure 1) from the Blood Vessel Analysis algorithm were imported into Definiens Developer XD 2 (Definiens AG, Munich, Germany) to remove vessel objects that cross the left or bottom line of the grid.

In the statistical program R, a script was written to calculate the intra-class correlation coefficient (ICC) (package ‘irr’, two-way model, type=agreement) and its confidence intervals (95%). R also was used to construct plots of the prediction interval.

**Results:** Accuracy of the algorithm was not only dependent on the sample, but also on the tumor type (ICC for CRC: 0.38, GBM: 0.04, OC: 0.23, RCC: 0.80) and in RCC, the level of topological heterogeneity (ICC for low: 0.92, medium: 0.77, high: 0.65).

**Discussion:** We presume that the dependency of the accuracy on tumor type was due to blood vessel architecture. For example, when algorithm-classified objects that cross the border of the region of interest were removed, the ICC for RCC was much lower (-0.16 vs. 0.80). It is conceivable that this is because of abundant vascularization in RCC. Indeed, when border-crossing objects were removed, most vessel objects were cleared away during the image analysis. Hence, this also occurs during manual analysis, but the image analysis is stricter.

**Conclusion**

The automatic algorithm needs to be further optimized. It is important to perform proper artifact detection and identifying necrosis before start of image analysis. The algorithm must be able to detect and classify different vessel types, but also varying vessel morphology due to sectioning or staining patterns. Validation of the results is needed.

**Computer-aided her2/neu evaluation in external quality control (EQA) of breast cancer screening programme**

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**Background:** Since 1998 in Veneto district, external quality assurance (EQA) is active for breast cancer. Since 2008, a slide scanner has been implemented for digital readings. The computerized algorithmic analysis Virtuoso software (Ventana, Roche, FDA cleared) has been used for evaluation of HER2/neu immunohistochemistry (IHC), in two groups of breast cancer, selected by the 25 Centers participating in the EQA. At the plenary session, virtual slides were available for discussion.

**Materials and methods:** The Centers selected 48 negative cases (score 0/1+) (group A) and 23 positive cases (score 3+) (group B) according to manual evaluation. Our team stained the slides with HER2 (PATHWAY anti-HER2 4B5) by Ventana BenchMark ULTRA staining system, automatically scanned by iScan Coreo (Ventana). The two pathologists selected the individual fields of view (FOVs) of the virtual immune slides, used Virtuoso Image Analysis application software, confirmed the results and generated the reports.

**Results:** The two pathologists confirmed the computer-assisted assessment of HER2 IHC assay with these results: in group A, 46 cases received 0/1+ score and 2 resulted not evaluable because of tissue artifacts in pre-analytical phase; in group B, 18 cases received 3+ score and 5 cases the 2+ score (the membrane staining was complete and intense within ≤10% of tumor cells). This evaluation completed inter-observer agreement.
All the virtual immune slides were evaluated during plenary session. 5 borderline cases obtained an heterogeneous interpretation after briefing.

**Conclusion:** The Virtuoso Image analysis Application Software assists the pathologist in the semi-quantitative measurement of HER2. In our study the computer-assisted analysis and the manual scoring were highly reproducible in HER2 negative (group A), with no false positive cases. The computer-aided assessment does help in screening negative cases reducing turnaround time. We found 25% disagreement in HER2 positive (group B), with underscore in Virtuoso software analysis (2+) compared to the manual one (3+). Overall, the computer-aided assessment decreases the inter-observer score discrepancies. The test results are only as good as the quality and accuracy of the IHC slide that is imaged, and the subsequent image that is analyzed. As recommended by CAP/ASCO, the system is an instrument that can aid the pathologists, however the final decision remains on the pathologist.

**Comparative Study between Quantitative Digital Image Analysis and Fluorescence In-Situ Hybridization (FISH) of Breast Cancer Equivocal HER2 Score 2+ cases**

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**Background:** Optimization of workflow for breast cancer samples with equivocal HER2/neu score 2+ results in routine practice, remains to be a central focus of the ongoing efforts to assess HER2 status. According to the College of American Pathologists/American Society of Clinical Oncology guidelines equivocal HER2/neu score 2+ cases are subject for further testing, usually by FISH investigations. It still remains an open question, whether quantitative digital image analysis of HER2 immunohistochemically (IHC) stained slides can assist in further refining the HER2 score 2+. 

**Aim of this work:** To assess utility of quantitative digital analysis of IHC stained slides and compare its performance to fluorescence in situ hybridization in cases of breast cancer with equivocal HER2 score 2+.

**Material and Methods:** Sixty specimens from breast cancer patients with previously (interactively) diagnosed represented the study population. Her2 stained slides were scored. Cases with HER2/new score of 2++, were digitally scanned by iScan [Produced by BioImagene (Now Roche-Ventana)]. The IHC signals of HER2 were measured using an automated image analyzing system (MECES, www.Diagnomx.eu/meces). Contemporary new cuts were prepared for FISH examination.

**Results:** Three out of the fifteen cases with equivocal HER2 score 2+, turned out to be positive (3+) by quantitative digital analysis, and 12 were found to be negative in FISH too. Two of these three positive cases proved to be positive with FISH, and only one was negative.

**Conclusions:** Quantitative digital analysis is highly sensitive and relatively specific when compared to FISH in detecting HER2/neu overexpression. Therefore, it represents a potential reliable substitute for FISH in breast cancer cases which desire further refinement of equivocal IHC results.

**FISHQuant quantification algorithm validation in the clinical molecular diagnostics**

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The revolutionary development of the information technology opens up new opportunities to all areas of medicine. The development of science provides more and more challenges, with which the traditional methods are no longer able to keep up. The microscope’s reports do not contain visual information about the sample, and do not provide for this possibility. The appearance of the digital pathology opens up new opportunities to the diagnostic, the research, and also to the education. The Fluorescence in situ hybridization (FISH) techniques to the fore, furthermore it ensures the safety of cytogenetic diagnosis, and not only for the metaphase cells but also for the interface cells. The FISH molecular techniques used in tumor diagnostics for example in the hematology and solid tumors cases, and are also used in the prenatal genetic disorders detection. FISH looks specifically at the one specific gene area of a chromosome only.

**Image Analysis:** Before a digital slide can be processed by the FISH algorithm, the user has to specify which fluorescent channel contains stained cell nuclei (usually stained with DAPI). First small but intense spots are detected in the
nuclei channel image and are used as seeds to grow the cell mask area, until the intensity in the image drops to a certain level or another nuclei's mask is encountered. In tissue samples bigger regions have to be considered for cutting (separation), which is applied recursively until the nucleus is small enough. The second step is the spot detection. Nuclei in the mask image and also spot channels are processed independently. The nucleus is masked out in the spot channel and intensity maxima (possible spots) are searched with an adaptive threshold algorithm, and then filtered by intensity. Spot areas sometimes have to be merged. The type of probe applied influences how merging is done. In case of HER2/neu probe spots in the amplification spot channel can form bigger connected clouds of strong signals. After processing each spot channel, fusion calculation connects spots from different spot channels into a fusion spot, primarily based on their distance. After image segmentation, geometric data such as nuclei and spot area, perimeter, and shape factor are measured. Finally clustering logic is applied. Empty nuclei and artefacts are also processed and put into special result classes.

The purpose of the investigation:
The biomarkers which labeled with DNA specific fluorescent dye are conquering in the routine diagnostic pathology. Our aim is to present our new developed FISHQuant quantification algorithm and compare the semi-automated image-processing results with the manually evaluated measurement on the same samples. We response to clinical cases, which identification of genetic variation of the FISH assay is part of the routine diagnostic procedures and which have a high degree of clinical relevance.

Test Process: The PANNORAMIC™ MIDI digital slide scanner used in the study, and Pannoramic Viewer 1.15.3. ver. software use to the digital slides appearance. The first step of the validation was the equivalence test between the conventional microscopic examination and the semi-automated FISHQuant. We selected clinical samples which contain numeric deviation for gene, for example p53 deletion in the hematology cases, or HER2 amplification in the breast cancer cases, and use structural deviation indicating probes, for example Philadelphia chromosomes (t(9;22)(q34;q11,2)) or t(8;21)(q22;q22) in the acute myeloid leukaemia. We examined the reproducibility of measurements of the FISHQuant algorithm. The samples that were previously analyzed by software have been evaluated, and after a few days later we measured again with the same software and we compared the results.
**B6: Computer Aided Diagnosis System**

**Frequential versus Spatial Colour Textons for Breast TMA Classification**

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**Spain**

**Introduction:** The use of TMA (Tissue microarray) generates large amounts of information, which requires careful analysis. The aim is to evaluate the TMA by automated methods in order to achieve an objective diagnosis in pathology. To this end, conspicuous texture features, called textons, have been used.

**Materials and Methods:** Once the breast TMA cores were digitalized at 10x, 628 representative regions of the 4 tissue classes were selected. The size of these regions was 200 x 200 pixels and the TMA tissue classes were: i) benign stromal tissue with low and medium cellularity, ii) adipose tissue, iii) benign structures and anomalous and iv) different kinds of malignity, that is, ductal and lobular carcinomas.

Classification is based on two different types of texton descriptors: spatial and frequential. The principal difference between the frequential and spatial textons is their responses. In frequential textons these responses are extracted by a filter bank whilst in spatial textons these responses are taken from an NxN square neighbourhood around each pixel of the original image. Besides, other aspects that have been considered are: colour models, the application of statistical features on the texton maps and the selection of a good feature set and a suitable classifier. Colour models used in this study were: RGB, CMYK, HSV, Lab, Luv, SCT and channel combinations: Lb and Hb.

**Results:** In this study, features were extracted by calculating the 1st and 2nd order statistics (or Haralick coefficients) on the texton maps for frequential and spatial texton. Besides, each feature set was extracted for a different colour model previously mentioned. Tissue classification was not only performed with each colour model individually, but also with the combination of several colour models. Besides, a Forward Sequential Search (FSS) method was selected to reduce the dimensionality of the feature dataset obtained a reduction of 72.56% of the initial features. Classification was performed by the AdaBoost classifier with 10-fold cross validation. Finally, the best result with frequentional textons was obtained by the CMYK&Hb&Lb&HSV&Luv&SCT combination with a classification error of 0.12 and 93.9% accuracy. On the other hand, for spatial textons the best result was obtained by the RGB&Hb&Lb&HSV&Luv&SCT combination with a classification error about 0.046 and 97.68% accuracy.

**Conclusion:** This study describes a complete study on breast TMA classification based on texton descriptors. A dataset of 628 TMA images divided into four classes was used. A suitable combination of colour models and features led us to achieve a 93.9% and a 97.68% accuracy using frequential and spatial textons respectively, making this study truly valuable in breast TMA classification. The AdaBoost classifier takes approximately 79 seconds to perform the training and the test in the classification.

**Computer-aided diagnosis from weak supervision: A benchmarking study**

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**Introduction:** Supervised machine learning is a powerful tool frequently used in computer-aided diagnosis (CAD) applications. The bottleneck of this technique is the demand for detailed expert annotations, which typically have to be very tedious for medical image analysis tasks. The multiple instance learning framework serves as a remedy to this problem by allowing labels to be provided for groups of observations. We quantify the power of existing multiple instance learning algorithms by evaluating their performance on two distinct CAD tasks: i) Barrett’s cancer diagnosis, and ii) diabetic retinopathy screening.

**Material and methods:** We include both standard and state-of-the-art MIL models in our set of methods to be evaluated. The list of MIL methods included in benchmarking is as follows: i) mi-Graph, ii) GP-MIL, iii) EMDD, iv) mi-SVM, v) MI-SVM, vi) Citation kNN, vii) MILBoost, viii) KI-SVM, ix) iAPR, and x) SIL-SVM.

**Results:** We evaluate the MIL algorithms on two use cases: i) Barrett's cancer diagnosis on a private data set provided by Institute of Pathology, Helmholtz Zentrum Munich, Germany, and ii) Detection of diabetes from retinography on the public Messidor data set collected by three universities in France. Among the models in comparison, mi-Graph provides the best...
performance in these two tasks with 86.4% and 72.5% accuracy, and an area under ROC curve (AUC) of 0.93 and 0.81, respectively.

Conclusion and discussion: The main outcome of the study is that the mi-Graph method generalizes best across application domains. Its closest competitor is MILBoost. It is noteworthy that SIL-SVM exhibits drastically lower performance than these two methods. This indicates that image-level supervision is weak, hence, the MIL formulation plays an important role in increasing prediction performance.

Automated identification of cell nuclei in tissue sections

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Introduction: For breast cancer patients, the sentinel lymph node status has been proven to be an important diagnostic factor. To accurately assess this parameter, detailed microscopic analysis of numerous tissue sections is required. As a first step towards automating this tedious and error-prone task, in the present study we developed an algorithm for automated identification and delineation of cell nuclei. Based on this analysis, diagnostically relevant features may subsequently be extracted. Identifying the locations of individual cell nuclei enables quantification of the spatial arrangements of cells within the tissue context. Furthermore, accurate automated delineation of cell nuclei enables quantitative analysis of nuclear morphology. These features are the major attributes in the characterization of cancer regions during automated prescreening of digitally scanned sections. Results of the method described here will be compared with previously published algorithms.

Material and Methods: Digitally recorded images (n=20) of hematoxylin and eosin (H&E) stained sections of eight different tissue types were used for algorithm development and validation. The images were preprocessed by a color deconvolution algorithm, to extract the nuclei signal. All nuclei present in these preprocessed images were manually outlined to obtain a set of ground truth segmentation masks. Automated identification of nuclei is performed using a randomized Hough transform for ellipse detection. Resulting ellipses were further refined using a globally optimal active contour model, performed on the obtained nuclear channel. Results of this approach were compared against the performance of other widely used segmentation algorithms, using the dice score.

Results and Discussion: Preliminary results demonstrate that the proposed approach has a higher success rate in detecting nuclei of more complex tissues, containing larger amounts of nuclear overlap. With the gain in accuracy, the computational burden also increases. Therefore, use of the newly proposed algorithm should ideally be combined with other approaches, yielding a hybrid approach with optimal quality/efficiency ratio.

Conclusion: We have developed an accurate approach for detection and segmentation of nuclei in digital images of H&E stained tissue sections, which can be used to extract features describing both the tissue structure and the morphology of individual nuclei for use in automated prescreening.

Application of Computerized Digital Image Cytometry of DNA Aneuploidy for Cervical Cancer Screening in China

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Introduction: Automated DNA aneuploidy testing by computerized digital image cytometry may play a role in population based cervical cancer screening in low resource areas/countries. We report a pilot study of a population based cervical cancer screening in rural China using digital image cytometry.

Subjects and Methods: From 2011 to 2012, 12,088 women from rural areas of Ordos City, Inner Mongolia Province, China, were included in the study, which was part of a city wide cervical cancer screening project approved by local health authority. Cervical cytology sample was collected to prepare a liquid based monolayer cytology slide, which was then stained with Feulgen stain. A computerized digital image cytometer was applied to automatically scan and digitize the images of cell nuclei on cytology slides and to analyze the digital images of nuclei for DNA aneuploidy of cells. Cytologic samples contained ≥ 3 cells exceeding 5c were considered positive for aneuploidy. Women with positive aneuploidy test were referred for colposcopy, and women with abnormal but not positive aneuploidy results were recommended for follow up in 6 months.
Introduction of a cancer tissue detection method via homology
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Introduction: Computer assisted pathological diagnosis is an important issue in the current situations of shortage of diagnostic pathologists. Many methods have been proposed so far, which can perform effectively has not been developed yet. This is because cancer tissue have too various forms, it is very difficult to apply the pattern recognition technology.

Recently, we have proposed a simple mathematical model to differentiate tumor tissues from their normal counterparts utilizing changes in the Betti numbers in tumorigenesis.

We may consider that the lesions, such as cancer, exist in the place where the contact degree is unusual area. Theoretically, the Betti numbers represent the degree of connection. We have calculated the Betti numbers each unit area for pathological samples taken in the virtual slide. Here we report the results and brush up points.

Methods: Morphological change in tumorigenesis and mathematical representation.

The concept of homology belongs to modern mathematics, and is not known in general. By using this concept, we can evaluate the contact degree between two points in the figure, quantitatively. The essential idea is that, because of the loss of contact inhibition of cancer cells, the contact degree of the cancerous lesions is different from compared with normal area. By calculating the Betti numbers per unit area. By calculating the Betti numbers per unit area.

In spite of the same tissue, they do not have exactly the same form. This makes difficult to apply the pattern recognition methods. In the homology theory, there is a concept of the topological invariance. The topological invariant is the quantity that is unchangeable by continuous transformation. The Betti numbers are one of the topological invariant. This is the reason why our results are robust.

The Betti numbers are the numbers coined after Enrico Betti, an Italian mathematician of topology. To understand the definition, we need expert knowledge of mathematics. However, in the two-dimensional case, it is very simple.

In two-dimensional case, the Betti numbers are consisting of two numbers. One is b0 (the 0-dimensional Betti number), which is the number of such isolated solid component as each cell or cell nucleus. The other is b1 (the 1-dimensional Betti number), which is the number of windows in the fenestrated area. These areas are created by incomplete fusion of neighboring isolated solid component.

Results: We have treated images taken at a magnification of 100 fold. Each tissue has its own characteristic size. The colon tissue case, the images are divided into 14×14 unit areas and the colored tiles are put on each unit area.

The numerical results indicate that the difference in our indices can differentiate tumor tissues from their normal counterparts. Because the integral of neutrophils and lymphoid follicles also have high contact degree, we have false positive. Combining some algorithms, we would like to reduce false positives.
**Quantitative assessment of liver steatosis using infrared microspectroscopy**

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**FRANCE**

**Introduction:** Liver transplantation is admitted as a curative treatment of end stage cirrhosis and hepatocellular carcinoma. However, the shortage of graft leads to use marginal organs. Thus livers with increased fat content also called steatosis are being used more frequently for transplantation, leading to an increased risk of graft dysfunction. The quality control of liver grafts relies upon the degree of steatosis. We have demonstrated that the correlation between histological examination and the concentration of triglycerides which are the major lipid species increased in steatosis, was poor ($r^2=0.54$). Therefore the incapacity of usual histological methods to rigorously provide an objective and non-biased assessment of steatosis strengthens the necessity to develop novel methods for the quantification of steatosis. We have addressed the potential of infrared microspectroscopy for investigating steatosis on tissue sections.

**Materials and Methods:** The study has been focused on liver samples obtained from surgical specimens including 10 normal livers and 25 steatotic livers. Frozen tissue sections stained with H&E were performed for histological examination. Steatosis was ranking between 5-60%. Adjacent tissue sections were deposited on gold coated glass slides for infrared microspectroscopy experiments. Acquisition was first performed with synchrotron radiation allowing investigations of the biochemical composition at the cellular level. Quantification of lipid content was performed by calculation ratio lipids/ proteins in the IR spectra from each pixel. The counterpart of each biopsy was used for lipidomic analysis leading to titration of triglycerides.

**Results and Discussion:** The correlation between quantification of the lipid content using IR microspectroscopy and triglycerides concentration was excellent ($r^2=0.92$). The method was further implemented on a laboratory microscope that allowed the objective quantification of steatosis on regular tissue sections performed at the hospital. This new method is rapid, not expensive and quantitative.

**Conclusion:** Quantification of lipid content in steatosis can be performed on tissue section using IR microspectroscopy. This approach that can be easily implemented into hospitals may open new avenues for rapid evaluation of liver grafts and diagnosis of fatty liver diseases.

**Performance of full-field optical coherence tomography (FFOCT) digital imaging for prostate cancer diagnosis**

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**FRANCE**

**Introduction:** There is a need for improved prostate cancer detection tools. Randomized biopsies lead to over-diagnosis and over-treatment, as well as unnecessary non-informative samples. Recent studies have shown that MRI-targeted biopsies allowed similar detection performance than randomized biopsies, while decreasing the number of cores. Full-field optical coherence tomography (FFOCT) is a new imaging tool creating digital images of biological tissues at ultrahigh resolution approaching traditional histological sections. It could be used to validate the cores just after they are biopsied, in particular when MRI-targeted.

**Material and Methods:** 116 fixed prostate biopsy cores from 38 patients were imaged with FFOCT shortly after the biopsy procedure, before they were sent to the pathology lab for standard histological assessment. Three pathologists were asked to analyze the FFOCT images to set reading criteria and provide a blinded diagnosis. The concordance between the FFOCT diagnosis and the histological diagnosis as well as the learning curve was recorded. A second reading session of all images was performed several months later using the finalized reading criteria.

**Results and Discussion:** Structures of normal tissue (eg fibro-muscular stroma, adipocytes, and vessels) could be recognized on the FFOCT images. The architectural details enabled to identify tumorous areas in the biopsies in many cases. The final accuracy predicted by the learning curve was over 80%, which was confirmed by the second reading session. The most frequent reasons for false negatives and false positives were small lesions and hyperplasia respectively.
Conclusion: FFOCT is as a fast and non-destructive novel imaging technique that provides a quick assessment of the tissue morphology. This study indicates that it could allow a good diagnostic accuracy for prostate cancer detection. The technique could be used on-site during the biopsy procedure to validate the biopsies and guide the number of biopsies to be performed, with a remote access to the pathologist expertise on the images.

The FourierScope - a New Whole Slide Imaging System Based on Fourier Ptychographic Microscopy

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UNITED STATES OF AMERICA, SINGAPORE

Fourier Ptychographic Microscopy (FPM) is a new method of producing images with high resolution and wide field of view without scanning the sample. Multiple wide-field, low-resolution images are collected using a range of illumination directions, and are then combined in the Fourier domain to produce the high resolution image. Even though the raw images collected are incoherent, the final image contains both intensity and phase data. Blurry raw images can be digitally refocused to produce a sharp final image through a very large depth of focus, and both chromatic and nonchromatic aberrations can be corrected within the algorithm.

We describe the method and algorithm of FPM, and show how it works. We also present a new commercial system, called FourierScope, which uses FPM to produce digital pathology slides. This system uses no moving parts and can produce a 12 mm diameter image with 20x resolution in less than 3 minutes. We compare FourierScope’s images with those of more conventional microscopes, both brightfield and phase. We also discuss the limitations of FPM and the prospects for improved performance of FourierScope in the near future.

Dry mass and cell cycle follow-up from Quantitative Phase Imaging

Julien Savatier, Sherazade Aknoun, Pierre Bon, Lamiae Abdeladim, Didier Marguet, Benoit Wattellier, Serge Monneret
FRANCE

Introduction: During cell cycle, a cell doubles all its components and divides into two cells. Cell cycle stage of individual cells is often studied with fluorescent labeling, by flow cytometry. Here, we propose a simple method to analyze cell cycle and dry mass fluctuations using quantitative phase imaging.

Material and Methods: The technique is based on a quadri-wave lateral shearing interferometry (QWLSI) wave front sensor [1]. It provides a quantitative measurement of the optical path difference (OPD = Δn·thickness) in nm, on each pixel of the image. This measurement, when integrated over the cell surface, is directly proportional to the cell dry mass [2], giving direct information on the cell growth. No labeling is needed. It is self-referenced and can be plugged on any microscope with classic objectives, a white light and a camera port. Since it is achromatic, it can be used in near infrared that cells do not absorb, for long live cell imaging. It can easily be combined with fluorescence for simultaneous correlative microscopy [3]. Automated segmentation of cells is easy thanks to the absence of halo or artifacts. It is fast (camera frame-rate limited) and sensitive (diffraction–limited in X and Y, ± 0.5 nm in OPD, ± 0.6 pg for a 570 pg cell).

Results and Discussion: After a complete metrology study, we analyzed different cell types, regarding dry mass and morphometric parameters, by single cell follow-up and population snapshot imaging. First, red blood cells as a standard since their dry mass is well known and not highly variable. Then two genotypes of two species of yeast (S. cerevisiae and S. pombe, haploid and diploid for each one), where we could identify significant differences between their dry mass. We studied also three different mammalian cell lines, under different conditions (control, starving and blocked in early mitosis by colcemid). The analysis of surface and dry mass, as well as qualitative parameters coming from the images, allowed us to define statistical values corresponding to stage G2 and M of the cell cycle. This gave us a mitotic potential which is important, especially for cancer studies.

Conclusion: The method is robust to record cellular division processes and effects of drugs or mutations on cell growth. The conjunction of automated segmentation, quantitative parameters and qualitative information coming from this fast, diffraction-limited imaging technique, as well as the possibility to use it simultaneously with fluorescent imaging techniques make it gainful for a lot of studies in biology or medicine.
Tissue imaging with quantitative phase imaging (QPI)
Sherazade Aknoun, Pierre Bon, Julien Savatier, Didier Marguet, Benoît Wattellier and Serge Monneret
FRANCE

Introduction: Immunohistochemical staining is now generally used to help diagnosis of diseases like tumors. Most of diagnosis tests involve clinical imaging at the cellular level performed by pathologists. While histology remains an excellent detection technique, its accuracy depends mostly on the expertise and interpretation of the pathologist.

We propose to use a new microscopy technique, demonstrated to be particularly suitable for measuring phase distribution of microscopic samples [1], to enhance contrast without any labelling so as to visualize the structures of the sample and to give objective criteria for comparing different tissues.

Material and Methods: The QPI technique is based on quadri-wave lateral shearing interferometry (QWLSI). It provides a quantitative measurement of the optical path difference (OPD) which can be directly linked to the refractive index and mechanical thickness of the sample. This OPD can be used as a new powerful source of contrast for unlabeled and unstained microscopic samples as it uses an intrinsic property of the sample itself.

The sensor enabling QPI can be directly plugged onto a non-modified conventional microscope like any camera using its native broadband light source.

Results and Discussion: It is known that in some particular cases like cancer developments, tissue reorganizations occur, characterized by significant morphological remodeling, more particularly of collagen network. The morphology and orientation of collagen fibers in the extracellular matrix have been shown to be an important reporter of tumor progression and can act as a predictive biomarker of a disease state.

We already showed OPD as a specific signature for some cellular components recognition like lysosomes [2], we propose now to use it as a morphological phase signature associated with tumor development.

Thin tissues imaging of few µm has been done and allowed structural comparison processing between different samples.

Another key advantage of our technique is that it can also be used to make tomographic reconstruction of thick tissues (80µm), under spatially incoherent illumination conditions. Optical slices can be created to visualize the 3D structure of the imaged tissue.

Conclusion: This new introduced imaging technique has shown its abilities to provide a great contrast of semi-transparent samples like cells and tissues.

The technique can be used for 2D and 3D imaging of a sample from few µm to 100µm thick, on a non-modified microscope, which allows its use in routine.

Images of tissues of mouse skin and tomographic reconstructions on mouse brain tissues will be shown.
From Microscopy, Imaging to Clinical Research: A Latin American Perspective
Alejandra Garcia, Eugenia Diaz, Bettina Müller, Härtel Steffen
CHILE

Advances in virtual microscopy, image processing, and digital pathology open new perspectives all over the world. In Latin America, most scientific, medical, and educational expertise is centralized in capitals and major cities, excluding the access of major parts of the population to state of the art education and medical services. Tele-medical/analytical tools, virtual imaging, or digital pathology open key perspectives to bridge these gaps. We present recent advances to provide high standard microscopic infrastructure, reproducible image processing code (IPOL/IPOL-LA), and access to high performance computing (NLHPC) via 10GB dark fiber within a Chilean Research Ring (U-BioMedHPC) to foster scientific and clinical perspectives for research, education, development, and services for the region.

Building upon the experience with a Center for Digital Sperm Analysis (www.cedai.cl), we are currently establishing the first Center for Digital Pathology in the region (CPDAI, www.microscopiavirtual.cl) which follows strategies to implement (i) a tele-interconsultant platform for second opinion, (ii) tools for quantification of lymphocytes in focal regions, (iii) the estrogen receptor in human breast tissue, (iv) the expression of Cerb2, and (v) patterns of fluorescent granules in kidney tissue with the Departments of Pathology and Oral Pathology of the University Hospital, public hospitals, and private clinics. Finally, we aim towards the integration of digital pathology data within clinical data and the first biobanks established within the region to explore new models for cancer research on a national and international level.

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functions classified the image, and postprocessing algorithms identified Ki67-positive and -negative tumor cells based on size, KL1 surrounding, and nuclear irregularity. The index was calculated in hot spots, global tumor areas, and invasive fronts. **Results and Discussion:** Manual indices were predominantly lower for double stains than single stains ($P<0.002$) indicating that double stains increased the accuracy of Ki67’s quantification. Precision was, on the other hand, unchanged by the choice of stain, given that the difference between observers was statistically significant for both stains ($P<0.001$). Pearson’s correlation coefficient for manual and automated indices ranged from 0.69-0.85 ($P<0.001$). Though some discordant cases were observed, we found the correlation acceptable. Firstly, the manual indices were rough estimates, and their validity as gold standard was thus indefinite. Secondly, the laboratory’s day-to-day variation for double stains was considerable, which caused difficulties in image analysis. When correlating automated indices with tumor characteristics, hot spots were slightly superior to other regions. Ki67 in hot spots correlated with tumor size ($P<0.001$), grade ($P=0.009$), and estrogen receptor status ($P=0.04$). **Conclusion:** Although precision was unsatisfactory for manual indices of both single and double stains, Ki67 should be quantified on double stains to reach a higher accuracy. Automated indices correlated with manual estimates and tumor characteristics, and they are possibly valuable tools in future exploration of Ki67 in breast cancer. When stains are standardized, the agreement between automated and manual indices should be further investigated, alternatively using virtual double stains.

**On-Demand Model for Digital Pathology**  
**Gerald Minkowitz, Jeremy Kagan**  
**ISRAEL, UNITED STATES OF AMERICA**

Advances in whole slide image scanning systems, improvements in high definition video and the effectiveness of remote microscopy, have lowered the barrier to entry of digital pathology across the globe. These factors, coupled with ever expanding access to the web have made digital pathology both affordable and readily available. Legacy models that require direct relationships amongst physicians, pathologists and hospitals are inhibiting the potential success of digital pathology as a remote diagnostic tool. Introduction of a new archetype will enable digital pathology to achieve its full global potential. This will enhance the level of medical care across developing and developed nations. The transformation of digital pathology into a tool that will enable all health care facilities, anywhere in the world, to supply and obtain expert pathology interpretations will benefit all medical facilities which have full, limited or non-existent pathology staff.

Our poster will present a comparative study of legacy and recent technological innovations. It will highlight the deployment of an on-demand model for digital pathology worldwide. This model relies on pioneering technology that will enable scheduling of on call remote diagnostic physician services via a web portal using a secure and trusted interface. It will provide identity verification, credential authentication, and real-time transaction processing. Our case studies show how this technology enables remote hospitals to provide their patients with expert diagnostics through their very own on call subspecialists. This model can be scaled for use.

**Digital pathology: a new tool in Pathology department**  
**Laurent Martin, Marie Hélène Aubriot-Lorton, Caroline, Damien Molly, Franck Ilgart, Brigitte de Boulard, Mathilde Funes de la Vega, Nicole Chapusot Laurent**  
**FRANCE**

**Introduction:** Digital pathology is an emerging tool that pathologists begin to include progressively into their daily practice. While our activity increases sharply and accreditation is about to enter our laboratory, automation of technical steps should allow the maintenance and amelioration of our workflow. In this context, production of digital slides would give pathologists new tools to interpret lesions, track pertinent data on slides and communicate more efficiently with remote pathologists or physicians. We report here our experience of digital pathology implementation in our Department located in a University hospital.

**Material and Methods:** After setting up new Laboratory Information Management system (LIMS, Diamic, Infologic) that allow technical tracking, a high-speed scanner (NanoZoomer, Hamamatsu) connected to Diamic via Calopix (TRIBVN) was installed. When slides are scanned, they are identified by 2D bar codes, indexed in Calopix and Diamic and sent automatically to a remote server (80 To). Then, virtual slides can be directly visualized by pathologists in Diamic after opening the file of the
exam or by using Calopix. Pathologists also have a remote access to virtual slides on all the computers of the hospital via the viewer Web pocket, provided the pathologist is connected with his/her user id.

**Results:** As scanning is time consuming, generating huge files (0.5 to 1 Go/slide) and human resources are not indefinite, digitalization of whole slides of the Department could not be performed. Then, only slides of interest, slides received or sent for expertise and slides used to qualify tissue for bio-bank and/or molecular pathology were selected for scanning. Digitalization of slides is prescribed in the LIMS by the pathologist as done for staining or immunohistochemistry. The working list, edited by the technician in charge of the scanner, allows the complete tracking of the process (digitalization, control of import and quality of virtual slide). Finally, the list is given to the pathologist as a reminder. In the lab, digital pathology is mainly used to quantify cells (percentage of tumoral cells), measurement (Breslow index in malignant melanoma for example) or to select region of interest (for macrodissection before DNA extraction). Outside the lab, sharing of digital slides in multidisciplinary sessions is highly appreciated by physicians and oncologists.

**Conclusion:** The integration of digital pathology within our Department of Pathology, in connection with the LIMS, is interesting because it allows for an automated and secure management of the whole workflow of the image. Even if its use is still limited and even if problems persist, it was welcomed well by all the pathologists of the Department. We are now thinking about the best way of integrating the images generated in research projects into the system.

**Multimodal biomarker study by PET and digital microscopy of the response to sunitinib on a luminal B-type mammary carcinoma model**

Benoît Theze, Nicholas Bernard, Audrey Beynel, Stephan Bouet, Bertrand Kuhnast, Bertrand Tavitian, Raphaël Beynel, Stephan Bouet, Bertrand Benoit Theze, Nicholas Bernard, Audrey Beynel, Stephan Bouet, Bertrand Kuhnast, Bertrand Tavitian, Raphaël Beynel, Stephan Bouet, Bertrand Benoit

**FRANCE**

**Introduction:** In vivo positron emission tomography (PET) is a powerful tool for assessing chemotherapy response in cancer research. Even with highly specific radiotracers, PET signal is affected by the tracer pharmacokinetics and by the tumour intrinsic features. Thus, PET imaging might benefit from additional information such as anapathology. Digital microscopy offers the opportunity to quantify the immunohistochemical labelling and extract morphological features of interest. This multimodal biomarker approach was used to evaluate the response to the sunitinib multi-kinase inhibitor on a murine syngenic orthotopic PyMT model of breast cancer.

**Material and methods:** With a neoadjuvant setting of 9 days sunitinib (40 mg/kg per os) vs vehicle administration, tumour sizes and body weights were monitored for each mouse group (n=4 and n=3 for treated and control respectively). Before and 6 days after the treatment course, the tumour metabolic activities and hypoxic levels were measured in vivo using [18F]-FDG and [18F]-FMISO PET tracers respectively. In parallel, a method based on ex vivo microscopy was developed to characterise the drug effects on vascular density (CD31), proliferation (Ki67), inflammation (F4/80), hypoxia (HIF1alpha) and tumour glucose metabolism (GLUT1). This method relies on immunohistochemistry, followed by an extensive numeric sampling by microscopy, and ended with an automated image analysis. This latter step used the ImageJ and CellProfiler open source software.

**Results:** Whereas mice body weights remained almost constant during the study, a 3.8 fold tumour size difference was measured by calliper between the treated and control groups (109 ± 24 mm³ (n=4) vs 418 ± 62 mm³ (n=3)). [18F]-FDG images revealed no significant changes on tumour/muscle signal ratios (T/M ratios) in treated (from 3.43 ± 0.73 to 3.35 ± 1.32, n=4) and control (from 4.29 ± 0.96 to 4.64 ± 0.63, n=3) groups. By contrast, [18F]-FMISO PET imaging showed a significant T/M ratio decrease (from 9.29 ± 0.90 to 5.11 ± 0.75, n=3, p=0.0035, **) in treated tumours vs a non-significant increase (from 6.34 ± 1.06 to 8.36 ± 2.17, n=3) in the control mice. Moreover, comparisons between treated vs control tumours revealed a 4.9 fold regression for vascular density (p=0.0219, *), a 2.03 reduction of HIF1alpha expression (p=0.0388, *), a 17.66 fold induction of apoptosis (p=0.0001, ***)**, a 1.83 fold reduction of proliferating cells (p=0.0464, *), a 1.71 fold reduction of macrophages (p=0.0426, *) and a 2.57 increase of GLUT1 expression (p=0.0213, *).

**Conclusion:** With an acceptable toxicity level, sunitinib induced tumour size reduction and pronounced anti-angiogenic and pro-apoptotic effects in our model. The comparison between in
vivo imaging and ex vivo microscopy brought additional information on the drug efficiency and clues on its mechanisms of action. The particularly high sensitivity of this model to sunitinib makes it relevant to further investigate the benefits/risks ratio focusing on patients bearing luminal B-type breast cancer. This work highlights the relevance of coupling PET and digital pathology analysis to monitor and understand tumour response to therapy.

**Acceptance of digital tumor board presentations in two medical institutions**

_**Dariusz Borys**_

**UNITED STATES OF AMERICA**

**Introduction:** Digital pathology is becoming more popular in research and clinical pathology. Tumor boards remain an important focus of pathology presentations in clinical pathology. We designed a research questionnaire to query the attitudes of clinicians regarding the accessibility and acceptance of digital pathology in the tumor board environment.

**Design:** We distributed survey during sarcoma tumor board at UC Davis and Pediatric Orthopedic Tumor Board at Shriner's Children Hospital. Survey questions included subspeciality, age, sex, and questions about satisfaction from digital tumor board compared to power point based presentations. Descriptive statistics were performed.

**Results:** The survey was completed by 18 of 25 respondents (72%), of whom 12 were male (67%). Specialties represented included orthopedic surgery (7/18), pathology (7/18), radiology (3/18), and surgical oncology (1/18). 50% were between 30-40 years old, while 33% were >40, and 17% were <30. All participants agreed or strongly agreed with the statement “I am satisfied with Digital Tumor Board quality”. Eleven respondents (61%) preferred Digital Pathology for Tumor Board presentations, 6 (33%) were neutral, and 1 (6%) preferred Powerpoint for Tumor Board presentations. All respondents agreed or strongly agreed with the need to incorporate Pathology and Radiology visual information into successful Tumor Board presentations.

**Conclusion:** Digital pathology is an accessible format for Tumor Board presentations which is widely accepted by diverse clinical specialities. Tumor Board presentations may be an optimal venue for the wider dissemination of this new and promising technology.

**Virtual slides versus binocular microscope use. An orthoptic evaluation of visual strain**

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**FRANCE**

**Introduction:** To compare the medical staff orthoptic visual complaints and changes with virtual slides and binocular microscope.

**Material and Methods:** A whole medical staff of Toulouse Rangueil University pathology department was recruited. An ophthalmological and orthoptic interrogation and examination were made before and after a day of work on virtual slides and binocular microscope (5-6 hours). Standard flat screens were used.

**Results:** Our study included 13 people (2 men, 11 women), mean age 40 years (26 to 63 +/- 9 years). Of the 13 subjects, nobody had tropia. After a working day on microscope, 63.6% of subjects had a near vision different deviation from the initial one, but 100% of subjects had changes after a working day on virtual slides. Motility was normal in all subjects.

No diplopia, limitation, nor hypo-hyperaction, nor alphabetical syndrome was observed before the work day or after work on screen or microscope. Punctum Proximum of convergence (PPC) is often changed, both after a day on microscope or computer, but not in the same way. After a day on microscope, PPC is increased whereas it is decreased with virtual slides. Changes in the amplitude of divergence vary after a day of work. After working on microscope, it is modified in almost all cases (after work on virtual slide or microscope).

All subjects had stereoscopic vision. Stereoscopic vision is generally not affected by the virtual slides. However, it varies in 12/13 cases among users of binocular microscope (increasing or decreasing).

Functional signs are varied (headache, watery eyes, eye redness, blurred vision, light diplopia, nausea, vomiting, periorbital pain, dry eye). After microscope working, only 2/13 cases complained of important functional signs against 8/13 after working on computer.

Discussion, **Conclusion:** To our knowledge this is the first publication about visual fatigue after use of a microscope or virtual slides. Our study shows that microscope use causes much less functional signs and visual genes than work on virtual slides and decompensates much less near vision than virtual slides work. Depth perception is modified.
after work on microscope but no after work on virtual slides.

**FlexMIm: towards efficient/effective Collaborative Digital Pathology**

*Philippe Bertheau*

**FRANCE**

**Introduction:** Nowadays, powerful telepathology tools allow reliable second opinion for difficult cases but still suffer from several weaknesses. Among them, the efficiency of the Whole Slide Images (WSI) display needs to be considerably improved in order to reach the one of the traditional microscopic examination. Besides, the overall WSI quality may be insufficient (blur…), necessitating rescanning and thus, workflow interruption. Due to these drawbacks, the potential for automatic analysis of huge amount of available digital files is still underexploited and the clinical adoption of telepathology tools is not yet a common reality. In this context, FlexMIm is a research project addressing these technological issues, in order to improve the telepathology and digital pathology processes.

**Methods:** FlexMIm consortium includes 27 pathology laboratories in the Paris region (coordinated by Assistance Publique-Hôpitaux de Paris), research laboratories from the Universities Pierre and Marie Curie-Paris 6 and Paris Diderot-Paris 7, as well as three companies: Orange Healthcare, TRIBVN and Pertimm. The project is based on cloud architecture (Orange Healthcare) embedding a dedicated WSI database and visualisation support (TRIBVN). Algorithms already developed for the WSI blur detection (Paris Diderot) will be integrated into the platform. The pathologists will use a test bed in order to evaluate compression algorithms on several visualisation devices (Orange Healthcare). Dedicated semantics will also be included in the platform, by supporting the Region of Interest (RoI) collaborative annotation in the targeted pathologies. These ontologies are issued from the contextual graphs structuring and modelling the pathology protocol (UPMC–Paris 6). The pathologists from the consortium will finally evaluate, on the platform, the semantic framework, the image collections and the image analysis algorithms developed during the project.

**Results:** FlexMIm project started in January 2013 and will last until January 2016. At this stage, we can assess:

- The blur detection module is integrated in the central server, being currently tested to improve the computational efficiency.
- The test bed for evaluating compression algorithms is now ready to be used by pathologists.
- An operational contextual graph has been produced and validated for the diagnosis of inflammatory bowel diseases (IBD).
- The semantic context linked to annotations of IBD WSI is under construction. Two additional pathological fields will be addressed using the same strategy: prostate biopsies and breast tumours.

Further works include the development of collections of fully annotated and retrievable reference images (companion images for the pathologist) and ultimately, the development of algorithms for an improved RoI detection in WSI.

**Conclusion:** This technological research project aims at optimizing the working time and comfort of pathologists, not only by reducing WSI displaying time with full plasticity of the interfaces, but also by giving the pathologists, online tools, able to help them to efficiently access RoI or automatically count for specific staining. These tools will then be used not only in the context of telepathology but also as online tools on shared platforms, for future daily practice of digital pathology.

Funding partners: BPI, ville de Paris, region île de France.

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**The TASTE* (Telepathological Assessment of Histopathological and Cytological Techniques) Project: Aiming to define European pathology slide technical standards**

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**ITALY, SWEDEN**

**Introduction:** Histological and cytological preparations represent the basis for several millions of pathological diagnosis per year in Europe. The diagnosis and the assessed of morphological prognostic and predictive parameter values are heavily dependent on the technical quality of the preparations. Yet, the technical quality shows considerable variations from laboratory to laboratory related to the level of education and dedication of the technicians, the instruments and reagents employed, and also the traditions at different places. Variation in technical quality of the preparations also
determines the quality of the virtual slides in digital pathology and impacts on reproducibility of the diagnoses in a telepathology scenario.

**Materials and Methods:** The TASTE project aims to define minimum technical standards by creating a web-based system in an ICT environment comprising a library of images of preparations being either of good technical quality or showing disturbing artefacts. The system is based on the open source software Moodle linked to a virtual microscope system, accessible through Internet, and on Olympus dotSlide system with NIS database; images are acquired at around 40,000/160,000 dpi.

**Results and Discussion:** Pathologists, residents, technicians and students from different countries can either learn from this image and description database or use the exercises with multi-choice answers for self-assessment. They can also submit microscopic images of their own preparations via the Web to a panel of internationally recognized experts for comments and suggestions. In addition to individual approach to the database, schools and laboratories will also be able to use the open system for education, tests, and quality control. Ultimately, it is expected that this innovative way of training will lead to establishing a TASTE virtual community and to the improvement of the technical quality of histological and cytological preparations in Europe.

**Automated image analysis is superior to manual reading of HER2 expression in breast cancer**

**Rossing HH, Talman MLM, Vainer B**

**DENMARK**

**Introduction:** Human epidermal growth factor receptor 2 (HER2) is a receptor for circulating growth factor, stimulating uncontrolled cell proliferation. The trastuzumab antibody reacts with HER2, arresting the cells in the G1 phase and presumably also inducing an immune system-directed cell killing. In breast cancer, overexpression of HER2 occurs in 15-20%, and analysis of HER2 expression is therefore pivotal for selecting the correct patients for this treatment. The aim of the study was to validate the digital, automated image analysis algorithm HER2-CONNECT™ (Visiopharm, Hørsholm, Denmark) which is developed to discriminate between amplified and non-amplified HER2 gene expression, with the final goal of minimizing the number of inconclusive 2+ scores. The validation is part of a general implementation of automated image analysis for routine breast cancer evaluation.

**Material and Methods:** Consecutive samples (n=315, from 157 patients) received at the department within the period week 23-34 were included. TMAs were routinely manufactured, and each core was reviewed in order to ensure the presence only of invasive carcinoma. Immunostaining (IHC) was performed with Dako’s ready-to-use HER2 test (Glostrup, Denmark). TMAs were scanned in a Mirax Midi Scanner (3D-Histech; Budapest, Hungary), and one batch analysis of the HER2-CONNECT algorithm was run including all samples. This algorithm evaluates the IHC staining reaction of HER2 based on cell membrane connectivity by using a dynamic measure (between 0 and 1) of the size of HER2-stained membrane fragments. The automatically read connectivity translates into the classic diagnostic score for HER2 protein expression (0, 1+, 2+ or 3+) in agreement with the ASCO/CAP guidelines. The automated reading was compared to manual reading of HER2 protein expression and for the borderline (2+) protein expression samples to manual reading of the HER2 gene expression on fluorescence in situ hybridization (FISH).

**Results and Discussion:** Manual reading demonstrated a sensitivity of 95.5% and a specificity of 81.0% with 17.0% inconclusive samples. Using the digital HER2-CONNECT test, both sensitivity and specificity increased (97.7% and 98.1%, respectively), and only 1.9% of the cases were deemed inconclusive. Total agreement when comparing HER2-CONNECT reading with manual IHC reading supplemented by FISH in borderline (2+) cases was 98.1%. Using the HER2-CONNECT digital image analysis algorithm based on IHC detection of HER2 protein expression on tumour cell membranes, less than 2% of the cases were inconclusive whether overexpressed or not.

**Conclusion:** Application of automated image analysis for HER2 protein expression instead of manual reading thus decreases the need for supplementary FISH testing by almost 90%. The impact on cost reduction and turn-around-time is obvious.
A segmentation method for images with subjective contours applied to immunohistochemistry-stained cell membranes

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CHILE

Introduction: Quantification of specific marker proteins in histological samples plays an important role in pathology field and basic research. Immunohistochemistry (IHC) is a technique that allows one to highlight the expressions of such proteins and to perform qualitative assessments of the tissue specimens. A typical case is the one of IHC images with over-expression of specific marker proteins on the cell membranes. This usually provides incomplete information about cell borders, leaving important portions of the membrane invisible. In many cases human observers can subjectively complete those invisible membrane portions (or “subjective contours”), but the use of computational methods are fundamental for a further objective quantification. Contour completion is an important challenge and diverse approaches have been proposed to address the segmentation of images with subjective contours, especially, deformable contours methods. However, these methods are mainly based on image gradient intensity making them prone to leaking problems in areas with low contrast or borders with missing information. We propose a segmentation method that allows one to segment the incomplete cell membranes present on IHC breast tissue images.

Material and Methods: The proposed segmentation method combines two main approaches: support vector machines (SVM) and subjective surfaces. Our hypothesis is that SVM and subjective surfaces approaches can be effectively combined in a robust segmentation method for images having subjective contours, in particular for segmentation of IHC-stained images. We choose SVM for segmentation of the visible membrane portions and the subjective surfaces approach for the reconstruction of the subjective contours. SVM is used as a pixel binary classifier. For each image, training and testing data were generated through the manual segmentation performed by a pathologist. The SVM result is the input of a modified subjective surfaces approach, which is being adapted for the IHC-stained image problem, in order to reconstruct the missing membrane portions.

Results: Currently, we have some preliminary results for segmentation of the visible membrane portions based on the SVM classifier. So far, the combination of various features including pixel edge and color saliency, CMYK, HSV and CIEL*a*b* color spaces were use to measure the classification performance compared with IHC-stained images previously graded by a pathologist. Discussion: The SVM generalization capability should provide robust results thanks to the extraction of descriptive pixel features that capture the complex characteristics of IHC-stained image and the generation of appropriate gold standard. The strength of the subjective surfaces approach for the reconstruction of complex shapes, can provide a robust segmentation for the problem of IHC-stained images. The main expected result is an automated segmentation method able to achieve comparable performance to segmentation by pathologists. Funding: FONDEF D11I1096, CCTVAL-FB0821

Comparison study between TIFF and downsampled images. Automated evaluation of cytokeratin-19 immunostained scanned breast cancer tissue microarray.

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SPAIN, POLAND

Introduction: High quality digital images (DIs) in non compressed Tagged Image File Format(TIFF) obtained from automated digital scanning of tissue microarrays (TMA) generate files of big size that require high-volume storage devices. To solve this problem several techniques to reduce file size could be performed. However, the automated quantification of immunohistochemically (IHC) stained markers could be affected in some standard compressed formats like JPEG. Low-resolution TIFF images obtained by downsampling process, which reduce the sampling rate of a signal in order to reduce data size, could also represent a good alternative. In this study, we compared the results obtained by automated image analysis quantification of the percentage of positive IHC stain between TIFF and the same downsampled images.

Material and Methods: 91 cores of 2 mm from ductal invasive breast cancer were included in TMA and stained with IHC for the cytokeratin-19 (CK19) marker. This marker is important for the
study of sentinel lymph node for the OSNA technique. Slides were digitalized with the scanner Aperio ScanScope-XT at 40X magnification. Each core from TMA was extracted as an individual DI in TIFF format. The low-resolution images were obtained by decimation of the original image calculated by downsampling an integer factor of 2. The final low-resolution DIs were 2 times smaller in each dimensions and 4 times smaller in image area and file size(110MB) than the original DI(442MB).

The automated processing method was developed using Fiji software. The first step evaluates the whole area of each core by using the Luminance channel, applying the median filter and gray-scale segmentation. The second step evaluates the number of positive objects stained in brown by IHC applying the Ruifrok A.C.(1997) method for obtaining a brown colour channel and then a gray-scale and size segmentation for positive objects, including holes inside of segmented area. The percentage of positive area of CK19 was calculated with the ratio between the pixels of positive objects and the total number of pixels of each cylinder. The agreement between the results obtained with the two types of DIs was evaluated with the intraclass correlation coefficient (ICC) and the Bland-Altman analysis.

Results and Discussion: The Bland-Altman analysis showed very small dispersion between the TIFF and low-resolution images and these differences were not influenced by the percentage of positive objects. Ninety-five percent of compared images have less than 4% of differences in the percentage of positive area. In 82 TIFF DIs the percentage of positive area of CK19 was higher than in low-resolution DIs. The ICC showed an excellent agreement(ICC=0.996) between both quantifications.

Conclusion: Despite high-capacity of data storage devices, image size reduction is still tested as an alternative to reduce the big size of the original DIs. The large number of DIs generated in research studies either by diagnostic techniques or as a result of the implementation of telepathology maybe a problem for some processing and storage. Low-resolution downsampling DI could be an effective method to reduce files size without compromise the evaluation of the percentage of positive stain of CK19.

Telepathology network in Ile de France: a 18-month experiment project for frozen sections (telediagnosis) and second opinions (teleexpertise).

FRANCE

Introduction: Because of health structure restructuration in France and shortage of pathologists with an increasing workload, there is a real need for new medical organization in pathology. The Regional Health Agency of Ile de France provided financial support to implement a Telepathology Network demonstrator. The aim of the experiment is to validate the medical organization and to prove the feasibility of Telepathology in the setting of a non dedicated information network.

Methods: This network includes 17 Pathology structures (11 academic hospitals, 5 general hospitals and 1 private) and 3 hospitals without Pathology lab. The project covers telediagnosis for frozen sections (4 binomials) and teleexpertise for second opinion diagnosis (all structures). Five general hospitals and the private structure are being equipped with a slide scanner (Hamamatsu) and a visualization and communication software (TRIBVN) completed by a macroscopy station (TRIBVN) and webconferencing software (Orange) for frozen sections. The academic structures are already equipped with scanners from different suppliers.

Results: The choice of a centralized platform (TRIBVN/Orange) will allow the workflow management, the fluidity of exchanges in a heterogeneous data-processing environment (working stations, network, scanners and format of virtual slides), the traceability and the evaluation of the telepathology activity. In a foreseeable future, this architecture could provide online tools for faster diagnoses and automatic counts of specific stainings.

The workflow will be different for frozen sections and second opinion consultation. For frozen sections, the technician is alone on the site of the surgical operation and can be assisted for gross examination and sampling by the remote pathologist through videomacroscopy. The technician performs cutting, staining and numerization of frozen sections. The virtual slides are available for the pathologist through a webconferencing triggered through the platform.
For second opinion, each case is registered on the platform and the requesting pathologist selects a consulting pathologist. Virtual slides are uploaded overnight on the regional platform to be available for the consulting pathologist. The main evaluation criteria will be: 1/ for frozen sections, the percentage of response times under 30mn and the proportion of frozen sections upon the total number of surgical operations 2/ for second opinions, the delay for an expert diagnosis through telepathology versus standard second opinion request and cost/efficiency ratio.

**Conclusion:** This telepathology experiment in Ile de France will allow to highlight and solve the technological locks, to evaluate the capacity of pathologists and technicians to adopt this technology, and to find the economic model.

**Image analysis in virtual slides: Comparison between the expression of hormonal receptors and DNA ploidy (static cytometry) in breast carcinoma.**

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**Mexico**

**Introduction:** Breast carcinoma is the most common malignant neoplasm in women and has an unpredictable clinical course. There have been some studies that analyzed several parameters that predict their biological behavior; one of them is DNA ploidy (quantification of DNA content). The aim of this study is to compare the expression of hormone receptors and DNA ploidy in breast carcinoma, using computer algorithms in virtual slides.

**Material and Methods:** This is a retrospective, descriptive and comparative study of 70 cases of breast carcinoma at The American British Cowdray Medical Center (Mexico City). All the cases in which hormone receptors (immunohistochemistry) and DNA ploidy (Feulgen stain) were performed were selected to be scanned using an iScan Coreo Au scanner (Ventana Medical System, Inc, Tucson AZ, US) at 20x magnification. The virtual slides were analyzed by one operator using SLIS and Virtuoso 5.1 software.

**Results:** A total of 70 cases were studied, 14 (20%) were diploid and 56 (80%) were not diploid, being the most common hypodiploid (44.29%), followed by aneuploid (28.57%) and tetraploid (7.15%). Depending on the histologic type, invasive ductal carcinomas were diploid in 26.53% of the cases, hypodiploid in 36.73%, aneuploid in 21.42% and tetraploid in 6.12%. Invasive lobular carcinomas were diploid in 10% of the cases, hypodiploid in 80% and aneuploid in 10%. Ductal carcinomas in situ were diploid in 9.09%, hypodiploid in 27.7%, aneuploid in 45.45% and tetraploid in 18.18%.

According to the hormone receptor expression, the cases positive to estrogen and progesterone receptors were diploid in 24%. The cases with positive estrogen and negative progesterone receptors were diploid in 16.67%. The cases with negative estrogen and positive progesterone receptors none were diploid, 40% were aneuploid, 40% hypodiploid and 20% tetraploid. The cases with negative estrogen and progesterone receptors 11.11% were diploid.

**Discussion and Conclusion:** In the present study we demonstrate that most of the tumor cells showed alterations in DNA content measured by static cytometry, with a predominance of hypodiploid cells. There was no significant relationship between histologic grade and DNA content; rather it was observed that in cases of ductal carcinoma in situ the neoplastic cells were mostly aneuploid.

**A Semantic Interoperability Framework for Facilitating Telepathology**

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**France**

**Introduction:** In current pilot telepathology projects, since non-standardized clinical information is exchanged, it is sometimes difficult for the anatomic pathology (AP) experts to capture the clinical context of the request. Exchanging standardized clinical information is hampered by the inability of AP information systems to integrate these standards while providing health professionals with adapted information input interfaces.

The objective is to propose a semantic interoperability framework for clinical information exchange between different AP departments that enables pathologists to use their own system and especially their local terminologies. The semantic interoperability platform aims i) to support electronically exchange of clinical information among disparate AP information systems while maintaining the meaning of the information being exchanged and ii) to provide to experts the clinical context
needed for the relevant interpretation of the request.

**Material and Methods:** We first designed the architecture of the semantic interoperability platform enabling the use of local interface terminologies by the pathologists. We modeled the complex telepathology workflow performed from the initial AP exam demand to report transmission, passing through advice request, access to shared/exchanges images, storage and analysis process.

We adapted the exchange transactions - LAB-35 and LAB-36 – defined by the IHE Inter-Laboratory Workflow (ILW) profile (1) to the context of telepathology. We proposed an ontological model (2) of the AP advice request based on HL7 v2.5.1 (3) and referring to the ISO/IEC 11179 and ISO 21090 standards (4). This model acts as a pivot model.

We developed services that enable i) the requester AP lab to transform clinical information of local request forms into the pivot model and to map local codes into standard codes from reference terminologies (5) and ii) the receiver AP lab to parse the request and transcode standard codes from reference terminologies into local codes.

**Results and Discussion:** The implemented prototype enables to send an AP advice request from a hospital A to a hospital B. In this prototype, we focused mainly on the Diagnostic Hypothesis and Clinical Information (problems, current treatment, and recent laboratory results) fields of the advice request form. The semantic services are used to transcode existing local codes to standard ones. For example, with regards to the Diagnostic Hypothesis information, some hospitals use locally the national coding system ADICAP (n=1 648 codes such as A7B1 - "lobular adenocarcinoma") while others use ICD-O (n=1181 codes such as M8520/3 - "lobular carcinoma"). During the transcoding process, SNOMED CT is used as the pivot reference terminology.

**Conclusion:** The lack of standards in telepathology exchanges hampers the interoperability between different AP information systems. Standardization and formal representation of clinical information in advice requests are necessary to improve the quality of the exchange and interpretation of clinical information. The implemented prototype was developed demonstrating the possibility to integrate telepathology functionalities into existing AP information systems used in daily routine by pathologists.

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**WSI virtualization: value added representation of Whole Slide Images**

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**Hungary**

Using Whole Slide Images gives several advantages in practice. During the last 10 years several articles and applications showed up from teleconsultation over the quantitative results of image analysis up to the full integrated pathology information systems.

All of these applications looked at WSI as a substitute function of the regular microscopy. Therefore WSI-s were used as a single entity as a regular slide is used and all requirements were pointing back to the regular microscopy.

Virtual objects created out of WSI are opening a new dimension in this field. The substance of the presented method is using the image information of the WSI as a starting point and creates a virtual layer over the images.

Essentially we set up two different ways of using WSI virtualization. The first is a kind of downscaling, when we use only a part of an existing WSI image and use it forward as the original source without harm or put any additional information into it.

The other way is the up scaling, when we merge the images into a common object and represent them in this way.

During the presentation we would like to show the benefits of using slide virtualization for TMA, education, research or clinical diagnostic purposes.

**Analyzing huge pathology images with open source software with an application to gliomas**

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**France**

Whole slide images (WSI) produced by digital scanners are becoming a routine technology for research, diagnosis, teaching and archival of patients' data. A major advance for clinical and biological research is the new access to high-quality quantitative data at the tissue scale and below, through computer-based analysis of the slides. Morphometric parameters of several hundred thousands cell nuclei can be measured.
Integration tools of the digital pathology system into the research biobank management solution

L Svanadze, T Franke, K Buckow, E Bahn-O Rienhoff

Introduction: Laboratory information management system (LIMS) has become increasingly popular in order to efficiently manage information about specimen. Microscopic examination plays a crucial role from the diagnostic point of view, but currently, pathology images are separated from the LIMS, and unlike radiology images, there are no standard guidelines about handling and integration of microscope images into the LIMS [1]. The work presented here is based on a research project for the Competence Network Multiple Sclerosis (KKNMS) that provides establishment of the digital pathology system (DPS) for researchers of KKNMS that integrates Multiple Sclerosis Brain Bank’s (MS-BB) high-resolution scans of histopathologic samples into the KKNMS IT infrastructure [2]. Objective of the work is to determine and evaluate effective configuration tools and mechanisms for the integration of the microscope images into an already existing web based biobank management solution.

Materials and Methods: The following steps were used to provide an efficient integration of DPS into the LIMS. At first, modules of the DPS were evaluated and key aspects like image server, storage infrastructure, web viewer, and client side requirements were analyzed. Second, general interface mechanisms were assessed by which the LIMS can interface with external system and an effective model of integration was defined for third party system where the linkage of digital slides to individual specimen was considered. Together all configuration tools derive concrete tasks for integration that were collected in the previous steps.

Results: DPS contains three significant components: image server, database, and web viewer for pathology images. We used Olympus dotSlide microscope for scanning of glass slides that additionally provides Net Image Server SQL platform to customers to manage images in a convenient way. Digital pathology images are much bigger than radiology images and it requires big data storage infrastructure. For example, if average image size is 2GB, 2TB of date storage holds approximately 700 microscopic images when configured in RAID 6 [3]. Customers can purchase separated high-performance server

automatically. In addition to sparing the pathologist this tedious task, it makes the analysis more repeatable. However, there are still issues in the everyday exploitation (e.g. on a laptop computer) of WSI. They typically occupy several gigabytes of memory and cannot be opened fully in a standard computer's memory. There is no standard format. Therefore, most common open source tools such as ImageJ fail at treating them, and the others require expensive hardware while still being prohibitively slow.

In the framework of the study of adult supratentorial diffuse low-grade gliomas (DLGG), we have developed several cross-platform, open source software tools to solve these problems [1, 2, 3]. Independent of proprietary libraries and very modular, they can be used in other open source projects. Their performance does not degrade when run with a modest memory amount (a few hundred MB) and compares very favorably to standard software.

They are able to transform microscopy images in the loosely supported NDPI format into one or several standard TIFF files, to create mosaics (division of huge images into small ones, with or without overlap) in various TIFF and JPEG formats, to access very quickly a small portion of a huge image. Some of the tools can be driven through ImageJ plugins, which make them user-friendly. They have been downloaded from more than 1500 sites (unique IP addresses) world-wide and are being used in production in several imaging platforms. New functions have been added continuously since their first release late 2012.

In our cohort of DLGG, they have allowed the quantification of several microscopic parameters of the brain tissue which feed mathematical models for personalized medicine, such as a new model of radiological treatment of adult low-grade gliomas [4]. These tumours extend beyond maximal visible MRI-defined abnormalities: a gap exists between the imaging signal changes and the actual tumor margins. We have shown by direct quantitative comparisons between MRI and WSI that the margins of T2-weighted MRI signal changes are mainly correlated with the edema fraction in tissue [5]. Through image analysis of WSI, we gained access to quantitative spatial density profiles of oedema, cell density and cycling tumor cell fraction. These profiles show striking patterns (e.g., the cycling cell density was higher at the limits of the MRI-defined abnormalities than close to the center of the tumor for 65.5% of patients).
hardware, or lots of companies on the market provide a cloud-based service. Digital pathology web viewer should provide viewing of metadata, navigation and annotation tools in conjunction with images. Most of the web-browsers are sufficient without installation in order to manage, view, and annotate digital images on the client side.

Most LIMS can provide toolset based on SOAP web services in order to integrate 3rd party system. After scanning process digital images are uploaded to the DPS server. Tissue samples registered in the LIMS has unique ID, and a URL and thumbnail of microscope image are registered for each tissue sample in the LIMS. DPS viewer is launching directly from the LIMS during the viewing of sample.

**Conclusion:** This study has shown integration tools of the DPS into the LIMS and the proposed method should be readily used in practice for KKNMS IT infrastructure. Integration of microscope images into the LIMS improves virtual access on images of specimen regardless of geographic diffusion of researchers which has clearly improved ergonomics as well.

Acknowledgements: this work was supported by the Competence Network Multiples Sclerosis (01GI1304B), funded by the German Federal Ministry of Education and Research.
Infrared spectral imaging to automatic assessment of tumor response
FRANCE

The rabbit Vx2 tumor is a fast-growing carcinoma model, which is commonly used to study different aspects of tumor behaviour under cancer treatments. The reduction of tumour viability and the degree of induced necrosis are the most common criteria to evaluate the efficacy of cancer treatments. The most recent developments in infrared microspectroscopy (IRMS) imaging aimed at automating the procedure of tissue recognition and quantification by using statistical methods and prediction algorithm. We used IRMS for the automatic characterization and quantification of the Vx2 liver tumor viability after a chemoembolization treatment.

Twenty-eight rabbits with Vx2 liver tumor were included in this study: 20 rabbits were subjected to a doxorubicin eluting beads (DEB) treatment and compared to a control (CTRL) group of 8 rabbits. The tumor bearing livers were resected, fixed in formalin and embedded in paraffin. Two adjacent sections were cut from each sample using a microtome. The first section was mounted on a calcium fluoride window suitable for IRMS imaging. The second section was put on a standard glass slide and stained with HES to serve as a control for IRMS imaging. On a first series of 14 different tumor sections (CTRL: 7 sections, DEB: 7 sections), we developed and validated a prediction algorithm. The protocol consisted of K-means (KM) clustering followed by linear discriminant analysis (LDA). The KM clustering was used to classify the spectra from the infrared images and to build a data base containing a large number of reference spectra (397,289 spectra) characteristics for tumor necrosis, viable tumor, fibrosis, liver parenchyma and liver parenchyma necrosis. Once the model validated, we empirically attributed a color for each type of tissue. Then, the predictive model was applied to infrared images of 52 new test tumor sections (CTRL: 9 sections, DEB: 43 sections). The result of the LDA model analysis is a false color image where each color corresponds to a type of tissue. The percentage of pixels corresponding to each color is automatically recorded by the LDA model. This advantage was used to calculate the mean percentage of surface occupied by each type of tissue and to determine the tumor viability after the DEB treatment. The LDA false color images reproduced the histological structures of Vx2 liver tumors. The sensitivity and specificity of the LDA model were high to 86.7% and 96.7% for the 5 tissue types respectively. For DEB group, the LDA model determined that the surface of necrotic tissue represented 77.68±23 % (CTRL group: 16.89±9 %, Mann Whitney: P<0.0001), the viable tumor 14.29±23 % (CTRL group: 74.74±7 %, MW: P<0.0001) and fibrosis 3.89±6 % of the tumor (CTRL group: 1.1±2 %, MW: P= 0.6262). The remaining percentage corresponded to unclassified spectra (DEB group: 3.94±7 %, CTRL group: 6.40±5 %, MW: P=0.0135).

Our results show that IR imaging coupled with LDA model analysis could be a helpful to easily assess tumor response.

3D quantitative histopathology in the mouse brain: from mesoscopic to microscopic scale
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FRANCE

Introduction: Histology is considered as a gold standard to investigate physiopathological markers in brain tissue sections but brain-wide quantitative analysis methods are lacking. We have developed an image processing toolbox that will be made available to the scientific community (BrainRAT toolbox in BrainVISA software: www.brainvisa.info). It is dedicated to the automated and reliable quantification of whole-brain histological datasets at the mesoscopic scale (a resolution of a few micrometers). Our method was initially tested for brain-wide mapping of mesoscopic histopathological abnormalities such as amyloid plaques (a neuropathological hallmark of Alzheimer's disease) and can be used to characterize mouse models of disease, evaluate new therapies at the preclinical stage, and validate in vivo small animal brain imaging techniques. We now aim at extending its scope to the microscopic scale to investigate (sub-)cellular markers in entire mouse brains.
**Material and Methods:** A brain of a transgenic mouse developing amyloid plaques was sectioned in the coronal plane. Series of interleaved sections (about one hundred sections per series) were stained for Nissl bodies, amyloid plaques and microglia. Sections were digitized with either Hamamastu NanoZoomer or Leica SCN400 F whole-slide imaging microscopes. We reconstructed the amyloid plaque histological dataset in 3D at a mesoscopic scale (a 4 gigabyte volume of 114 section images with a lateral resolution of 3.6 μm each), segmented amyloid plaques with a color-based machine learning algorithm and generated a 3D brain-wide quantitative density map of the amyloid plaque staining. In addition, we segmented one high-resolution section image for all the series (lateral resolution: 0.25 μm) using a parallelized version of our algorithm and generated 2D whole-section quantitative density maps for each staining.

**Results and Discussion:** Our toolbox accurately reconstructed a whole-brain dataset and successfully quantified a histopathological marker (amyloid plaques in this case) at the mesoscopic scale. In addition, we segmented cellular markers (microglia, phagocytic microglia, Nissl staining) and amyloid plaques at a microscopic scale on high-resolution histological images of the whole sections. Interestingly density maps derived from adjacent histological sections stained for amyloid plaques and microglia show a similar distribution pattern of those markers. This result is consistent with previous studies showing that microglial cells converge to amyloid plaques in Alzheimer's disease. Segmentation of high-resolution images could be performed in a few minutes thanks to parallel computing. Further developments are needed to adapt our software in order to reconstruct and quantify high-resolution brain-wide histological datasets of several terabytes and to extract relevant biological information. Quantitative density maps derived from high-resolution 3D histology will allow correlation of multiple markers throughout entire brains of mouse models of neurological disorders and possibly enable to better understand underlying physiopathological mechanisms.

**Conclusion:** BrainRAT can be used yet to reliably and extensively quantify histopathological markers in the mouse brain at the mesoscopic scale. Storage and processing of large datasets remain a challenge but the combination of high-resolution whole-slide imaging and BrainRAT is paving the way for large scale studies investigating (sub-)cellular biomarkers in entire mouse brains.
American Society of Clinical Oncology/College of American Pathologists.
The concordance between the two patterns of evaluation from image analysis and between immunohistochemical and FISH results was determined by the percentage of concordance and the kappa index.

Results: There was an excellent concordance in the image analysis of HER2 by immunohistochemistry in five and ten fields of view using single-click rectangle analysis (96%, kappa = 0.94). There was very good agreement between the categorized immunohistochemical (3+ (positive) and 1+, 0+ (negative)) scores and the FISH results (87.5%, kappa= 0.86). The FISH results were positive in 77.77% and negative in 22.23% of cases with equivocal results (2+).

Discussion and Conclusion: In the present study, we applied fully automated image analysis to define HER2 scores by immunohistochemistry in two patterns, and we demonstrated that the concordance is almost perfect. When we compared the results of immunohistochemistry with those from FISH, the negative and positive cases had good concordance; however, the equivocal cases showed a high percentage of overexpression that was detected by FISH.

In conclusion, the study of five fields of view with single-click rectangle analysis is an effective method to determine the HER2 score, and there is a good correlation between immunohistochemistry and FISH in unequivocally positive and negative cases. All of the equivocal cases defined by immunohistochemistry had a high tendency to be determined as positive by FISH.

Typing less common ovarian tumors: A training tool based on a pattern-based algorithm applied to a set of 20 virtual slides.

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Context: Ovarian tumors (OT) typing is a competency expected from pathologists, with significant clinical implications. OT however come in numerous different types, some rather rare, with the consequence of few opportunities for practice in some departments.

Aim: Our aim was to design a tool for pathologists to train in less common OT typing.

Method and Results: Representative slides of 20 less common OT were scanned (Nano Zoomer Digital Hamamatsu®) and the diagnostic algorithm proposed by Young and Scully applied to each case (Young RH and Scully RE, Seminars in Diagnostic Pathology 2001, 18: 161-235) to include: recognition of morphological pattern(s); shortlisting of differential diagnosis; proposition of relevant immunohistochemical markers.

The next steps of this project will be: evaluation of the tool in several post-graduate training centers in Europe and Québec; improvement of its design based on evaluation results; diffusion to a larger public.

Discussion: In clinical medicine, solving many cases is recognized as of utmost importance for a novice to become an expert. This project relies on the virtual slides technology to provide pathologists with a learning tool aimed at increasing their skills in OT typing. After due evaluation, this model might be extended to other uncommon tumors.

Virtual Slides for Teaching Pathology & Hematology
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Introduction: A virtual microscope or a virtual slide simulates the experience of examining a glass slide under the microscope. Briefly, virtual microscopy involves digitizing a tissue specimen on a glass slide, processing and archiving the images on a server and viewing them on a monitor with a browser. The interactive manner by which images are viewed (panning, zooming in and out) mimics the experience of examining a glass slide under an optical microscope (Fontem et al. 2005)

Material & Methods: An Olympus BX51 microscope was used. The images were captured using an Olympus CX10. The camera was connected to a PC Pentium 4 2.53 GHz computer. DotSlide image acquisition and Olyvia microscope control software were used to capture and view the images.

We evaluated the diagnostic accuracy of a virtual microscopy setup using surgical pathology specimens and hematologic malignancy in a university hospital setting. We discuss the development process and its potential applications in medical education and telemedicine.

Results & Discussion: Our first project was supported by ESF and state budget of Czech
Republic through the Operating Program Education for Competitiveness provided by the Czech Ministry of Education Youth and Sports. The main aim of the project is to improve the education of hematologic microscope morphology using digital virtual technology and simultaneously to verify the improvement of hematologic morphology knowledge in target groups. The second project Innovation of education microscopic morphology of pathology was supported by Czech Ministry of Education, Youth and Sport. The electronic database of 150 rigorous pathologic specimens was created. The database and technologies specific pathologic-medical diagnosis will serve for further educational needs.

Conclusion: This work was supported by grant No CZ.1.07/2.2.00/07.0294, 201100191/10, and project Biomedicine for regional development and human resources (BIOMEDREG) C1.05/2.1.00/01.0030.

Computer-assisted diagnosis of malaria infection with an automated microscopy system
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GERMANY

Introduction: Malaria is an infectious disease with high incidence in tropical and subtropical regions. Approximately 240 million cases and about 800,000 deaths were reported in 2012. Five species of the genus Plasmodium are responsible for malaria infections: P. falciparum, P. vivax, P. ovale, P. malariae and also P. knowlesi a Plasmodium species originally thought to be restricted to macaques. These parasites can be detected and classified by microscopic examinations of stained blood smears which represent the diagnostic gold standard recommended by the WHO. To diagnose malaria the so-called thick smear is used for the initial detection of malaria parasites. In addition to that, thin smears are used for species confirmation. As the process of slide reading under the microscope is an error-prone and tedious issue the Fraunhofer IIS is developing a computer-assisted microscopy system to support detection and diagnosis of malaria. This work summarizes preliminary results on the classification in thin smears.

Material and Methods: Central to this work is a collection of blood smears that have been drawn from routine work by the Bernhard-Nocht-Institute (BNI). These smears have been digitized at high magnification using a 100x oil immersion objective with an NA of 1.3 and a CCD camera (6.45 μm pixel size, 1000x1000). On this database of images an algorithm was applied to detect and segment erythrocytes and plasmodia which subsequently were pre-classified by trained personnel. For this work parasite infected and non-infected erythrocytes with artifacts have been differentiated.

Different color texture algorithms have been used to characterize the samples by quantitative features for machine learning algorithms such as support vector machines (SVM).

The SVM classifier has been trained on a training database of 400 erythrocytes with artifacts and 1197 erythrocytes with plasmodia. For the evaluation a disjoint test database of 829 and 2985 non-infected vs. infected erythrocytes has been used.

Results and Discussion: The total accuracy reached on our test database has been 93% with a sensitivity of 94% and a specificity of 90%. Annotation of erythrocytes has been validated by a clinical expert at BNI with substantial experience in malaria slide reading. Using our approach it is possible to discern non-infected erythrocytes with artefacts from infected erythrocytes. The algorithm may be expanded to other developmental stages of the parasite. However, for P. falciparum, the most prevalent species, ring stages account for the majority of cases.

Conclusion: The work presented here is an important step towards an automated malaria diagnosis in case an infection has been confirmed in the thick smear. Aiming at a complete system including parasite detection we are currently developing an automated scanning platform called SCube for microscopy that is working with automated slide loading and an oil immersion unit. The next step will be to integrate the algorithms into the workflow of scanning, detection and classification. With such a system diagnosing malaria infections should become a less tedious, secure, reproducible and even more objective process. Better quality assurance, improved documentation and global data availability will be additional benefits.
Full-field optical coherence tomography (FFOCT) of breast tissue: a new diagnostic tool for evaluation of breast proliferations?

Ana Tereza Nadan, Eugénie Dalimier, Vincent Servois, Brigitte Sigal-Zafrani

Introduction: Full-field optical coherence tomography (FFOCT) is a real-time imaging technique that generates high-resolution three-dimensional tomographic images from unprocessed and unstained tissues. Lack of tissue processing and associated artifacts, along with the ability to generate large-field images quickly, warrants its exploration as a complementary diagnostic tool. We used full-field optical coherence tomography (FFOCT) to image human breast tissue to assess its ability as a complementary diagnostic tool.

Materials and Methods: In order to improve the results already obtained in one pilot study, where the FFOCT images were classified as benign or malignant, we classified the alterations of the FFOCT images in two categories: atypical glandular proliferation (AGP) and non-atypical glandular proliferation (NAGP). Forty-five specimens from thirty-nine patients who had breast lesion assessed by biopsy or surgical resection were collected. They were imaged with FFOCT after fixation and then submitted for routine histopathology. Two blinded pathologists independently rendered diagnoses based on FFOCT images. Histological slides were scanned and compared with the FFOCT images for correlation and comparison.

Results and discussion: Normal breast architecture (adipocytes, fibrous stroma, lobules, ducts and blood vessels) was readily identified with FFOCT. Using FF-OCT images alone, the two pathologists identified atypical glandular proliferation in 31 cases and no atypical proliferation in 5 cases. After comparison with histological slides, the sensitivity was of 89% (31/35) and specificity of 60% (6/10) respectively. Disagreement was observed in eight cases. Four cases with histological normal breast parenchyma were incorrectly classified as AGP by FFOCT, whereas four cases with atypical proliferations were incorrectly classified as NAGP by FFOCT.

Conclusion: FFOCT of breast tissue is feasible and allows concordant results with those of pathological analysis in the majority of the cases, but the marge of error needs to be diminished for clinical purposes. We posit that greater pathologist experience, complemented with morphometric analysis and color-coding of image components, may help minimizing the contribution of these confounders in the future.

Evaluation of classical and virtual slide teaching methods of practical histology

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Introduction: The e-learning format of histology practical based on virtual microscopy provides many benefits over classical observation of glass histology slides. We have developed and applied a new format of histology practical with virtual slides observation and photography. Additional supporting documents were also available for each of the histology topic. Students evaluated positively the use of PC (personal computers) for observation of virtual slides and teachers benefited from a uniform quality of presented slides and also from a straightforward and subject-focused communication with students. In this report we present evaluation of both, the classical and computerized teaching sessions.

Methods: We have designed a questionnaire aimed at student’s experience with virtual and classical histology teaching sessions. This questionnaire was presented to students of the 2nd year of General Medicine and Dentistry programs that already passed normal histology course with PC-based practical and to the 3rd year students that in their previous year experienced histology course with PC-based practical and were currently attending histopathology practical in classical format of glass slide presentation. All together, 224 students of the 2nd year and 152 students of the 3rd year responded to this survey.

Results and Discussion: At the end of the course, 97% of the 2nd year histology students unequivocally preferred virtual microscopy as their favored slide observation method. Some of them (33-62%) occasionally combined both the glass and virtual slide observations. Although 46% of the 3rd year histopathology students experienced some discomfort when they switched to manual examination of glass slides, 51% of responding students still preferred virtual microscopy, 45% opted for a mixed observation of virtual and glass slides, and only 4% of respondents preferred the classical microscope observation method. Students also appreciated their active learning during observation of virtual slides, as they could take digital shots of important parts of displayed images and create their own presentations for later revisions. This corresponds to the survey in which 69%
histopathology students replied that they use their histology presentations for revisions in subsequent morphological studies.

**Conclusion:** Based on this survey as well as on teacher’s experience, we are concluding that the PC-based practical teaching method is attractive and beneficial to today’s computer - trained generation of future medical professionals. These students are able to utilize fully the capability of modern PC technology and absorb information from new software applications easily. They are also capable to create their own presentation records of observed structures that can be used in later revisions or seminars. In this way students are also educated in making scientific records and their application to professional reports or dissertations.

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**Discrepancies between diagnoses of real and virtual microscopy compared with intra and inter-observer’s variation**

Ichiro Mori, Yoshiyuki Osamura

JAPAN

**Introduction:** Virtual slide (VS) primary diagnosis becomes real issue based on recent development of digital pathology technology. There still exist objections or arguments to make primary diagnosis using VS. To clarify the issues of VS primary diagnosis, we made comparison experiment between virtual and real microscopic diagnosis.

**Methods:** Two independent comparison studies were made using different VS scanners using pathology files of Mita Hospital one year before. Large operation specimens were omitted. First, 119 cases composed of 154 fragments were scanned by NanoZoomer-XR (Hamamatsu). Second, 99 cases composed of 162 fragments were scanned by Aperio AT2 (Leica). These VS were independently diagnosed by 2 pathologists, and compared to the original diagnosis.

**Results:** We found 14/154 minor discrepancies in 1st experiment, and 20/162 in 2nd experiment. About the discrepancies between real and virtual microscopy, there are 2/154 and 5/162 cases that 2 pathologists made same diagnosis by VS which were different from the original diagnosis. On the other hand, there were 7/154 and 15/162 inter-observer’s variation and 5/154 and 0/162 intra-observer’s variation. Most frequent discrepancy was found in the grade of colon tubular adenoma. Grade of uterine cervical CIN and grade of oral to esophageal squamous epithelial atypia followed. There also were discrepancies between interpretation of atypical breast intraductal papillary lesion, gastric atypical glands, and possible MALT lesion. The tendency is nearly the same between the 2 scanners.

**Conclusion:** VS keep good enough quality to make primary diagnosis. There were more inter and intra-observer’s variation than virtual and real microscope discrepancies. Pathologic grading of colon adenoma or uterine cervical CIN is to categorize the continuously transforming morphological features into several grades. So, narrow gray-zone always remains on the boundary, and perfect match is basically hard to accomplish. When comparing virtual and real microscope diagnosis, it is important to pay attention to inter and intra-observer’s variation.

**Teaching Anatomical Pathology at the University of Barcelona: Transition to Virtual Slides and Virtual Microscopes**

Oriol Ordi, Josep Antoni Bombí, Antonio Martínez, Josep Ramírez, Llúcia Alòs, Elena Gonzalvo, Paola Castillo, Miriam Cuatrecasas, Teresa Ribaltà, Pedro L. Fernández, Jaume Ordi

SPAIN

**Background:** Digitized slides produced by whole slide image (WSI) scanners can be shared over the Internet and visualized on computer monitors or mobile devices. WSI can overcome many of the disadvantages of using glass slides for teaching purposes, including expense and restricted access to microscopes, limitations of the type of glass slides that can be shared, and loss of their stain quality over time. As a result, WSI has begun to replace the traditional light microscope, offering many opportunities for education. Moreover, this technology should be considered the implementation in education of a new tool that will become the standard in routine diagnosis in the next future. However, the creation of digital slide teaching sets requires mechanisms to make the images available to the students as well as software to manage the files and link them to informative data.

**Methods:** Two independent groups taking the discipline of Pathology in the same course, one of them using CM and the other WSI, were evaluated. The same set of H&E slides used for the CM classes was digitized in a Ventana iScan HT at 20x and observed by the students with the
Posters – Technology Advances, E-learning, Quality, Computer Aided Diagnosis

12th European Congress on Digital Pathology, Paris, France; 18-21 June 2014

Virtuoso viewer (Roche diagnostics) as navigator. The students access the virtual slides through a hyperlink on the open-source course management system Moodle (modular object-oriented dynamic learning environment). The educational text discussions are available on this platform as pdf files. The scanned images can be viewed up to a magnification of 400x at any time and in any place using the Virtuoso viewer (Roche) and the students’ computers as virtual microscopes. The skill level reached by the students was evaluated with an online test based on pictures. Day and hour of any single accession to the WSI by any of the students was registered. A voluntary survey was undertaken to the WSI group to assess the students’ impressions regarding the resource.

Results: No differences were observed between both groups in their marks in the online test (mean marks for the CM and the WSI groups: 9.87 ± 0.34 and 9.86 ± 0.53, respectively; p=0.880). 86.6% of the students found the software friendly, easy-to-use and effective for the purposes of the course. 71.6% of the students considered navigation with WSI easier than with CM. The most appreciated feature of WSI was the possibility to accede to the images anywhere and at any time (93.3%). 57.5% of the accessions were made on holidays and 41.9% later than 6:00 pm.

Conclusions: Digital WSI is a powerful educational tool that effectively replaces the traditional methods of teaching and learning pathology. It provides mobility and convenience to medical students. Virtual microscopy allows educators and students to take advantage of the accessibility and pedagogic versatility of the computer and the Internet.

Completely automated integrated system for prostate cancer grading
Luca Molinaro, Valentina Pompa, Filippo Molinari, Anna Sapino
ITALY

Introduction: Prostate cancer is the most frequent type of neoplasia in adult male population representing 20% of all tumours. In 1966 Donald F. Gleason created a unique grading system for prostatic carcinoma designed on a histopathological study of 270 patients from the Minneapolis Veterans Administration Hospital. This system is based on tumour architectural growth pattern (type of glands, size, distribution) and is composed of five architectural patterns including all type of growth pattern of prostate carcinomas. Few improvements have been made since then.

Although clear morphological aspects are described for Gleason score, interobserver reproducibility is high but not absolute, in particular between 6 (3+3), 7 (3+4 or 4+3) and 9 (5+4 or 4+5) categories. This is mainly due to difficulty for most pathologists to substitute old mental image libraries with new concepts, and in part by impossibility to define exact size and quantity of glands (i.e. “small glands” “few glands”, “large sheets” etc).

Material and Methods: A collaboration with Department of Electronics and Telecommunications (DET) of Politecnico di Torino was set up in order to design an automatic and computerized algorithm for Gleason Score assessment.

Ten cases of resected prostate carcinoma were selected from San Giovanni Battista Molinette University of Turin Hospital files, with different Gleason scores, i.e. two cases for each score: 6 (3+3), 7 (3+4 or 4+3), 8 (4+4) and 9 (4+5). All cases were reviewed by an expert pathologist and overall Gleason score was confirmed. Hematoxylin and Eosin slides were scanned using Aperio Scan Scope XT at 20x magnification. Several representative fields at 8x digital magnification of pure 3, 4 and 5 patterns were captured from each slide and all pictures were reviewed by the pathologist in order to quantify and determine Gleason score for each shot.

Representative images of pure 3, 4 and 5 patterns were analyzed to train the algorithm considering also morphological variants for each pattern (i.e fused glands and cribriform structures in patten 4). A step of pre-processing using color deconvolution protocols was achieved by splitting RGB images in three channels (one for Hematoxylin, one for Eosin, and background) in order to obtain gray scale images. Morphological operators and texture analysis were used to identify five object classes: nuclei, cytoplasm, lumen stroma and non-interesting objects. The algorithm was designed using Matlab.

Results and Discussion: The algorithm succesfully segmented and classified prostate cancer structures in their different patterns. In particular, it successfully discriminated pure pattern 3, cribriform structures of pattern 4 and pattern 5 either as “solid sheet” or “single cell infiltration” architecture with no errors. Less successful results were observed when discriminating between pattern 3 and glandular pattern 4 with “Fused microacinar glands” features.

Conclusion: Automated image analysis architectures with development of specific
algorithms represent promising tools that are likely to help standardize tumour pathology evaluation.

**Using a rich internet application to teach histology**


**Belgium**

**Introduction:** In 2012, medical studies in Belgium have undergone an important reform. At the University of Liège, the changes lead to a rocketing rise of the number of students, inducing serious problems regarding the management of the practical sessions in histology. The team of teachers took this renewed context as an opportunity to thoroughly revamp their teaching methods. A strong eLearning component has been set up, stimulating both students’ acquaintance with digital microscopic images and autonomous learning. An existing, web-based, image storage and analysis platform [Marée et al., 2013] called Cytomine (http://www.cytomine.be) was selected to host pedagogical resources and activities. The articulation between individual work, face-to-face course and practical sessions in labs has been revised in search of a stronger convergence. A pilot group of students has tested the eLearning tool in December 2013 before their exam. This abstract a) describes the instructional design of the blended learning setting with an emphasis on how the professional platform was accommodated to training purposes, b) reports about aspects of the student’s experience with the new tool.

**Material and Methods:**

**Sample and schedule:** the subjects are 270 students of third year who had access to the tool from 20 December to their exam one month later.

**Course:** the course is special histology. It aims at making students familiar with the fundamentals of organs and tissues identification and comparison.

**eLearning component:** The user interface of the Cytomine web platform was simplified and new web services were developed to make it appropriate for training purposes. It hosts for this course 50 histological sections. Students were mainly confronted to two types of learning activities: exploration and annotation of digital slides. They were requested to use the tool in supplement of regular courses and face-to-face sessions.

**Measure instruments and data type:** an ad hoc questionnaire has been designed to collect students’ feedback on their experience with the new method and tool: usefulness and limitations of the eLearning component, sense of learning, perceived convergence with the course, etc. This qualitative data will be related with students’ score at the exam which followed the eLearning sessions, and with basic behavioral metrics automatically extracted from the Cytomine platform in order to outline study traits.

**Results and Discussion:** By the time this abstract is submitted, the exam is on its way. It is therefore impossible to provide genuine results. Some preliminary assumptions can nevertheless be made, based on tutors’ experience and observations while coaching the eLearning session. The tool seems to have been welcomed by the students. Most of the exercises were duly performed and calls for help were kept limited. It advocates for a satisfying interface and task definition. It will be the work of the full-fledged analysis to nuance this perceptual data.

**Conclusion:** Based on a combination of self-reported data, performance records and interaction histories obtained through data mining, this preliminary and ongoing work explores students’ perceptions and training behaviors when experiencing an integrated eLearning module. Documenting such an innovative setting opens promising avenues to address issues related to the transformation of teaching/learning methods in medical education.

**Virtual slides for continuing medical education in pathology**

J.-Y. Scoazec, S. Prevot, B. Fabiani, E. Poullier, C. Guettier

**France**

Because of an increasing workload, the disposibility of pathologists for continuing medical education (CME) is reduced. The virtual slide technology offers them the possibility to attend CME sessions from their own lab or at home. The French Society of Pathology (SFP) has organized since 2012 an annual e-training course on liver biopsy based on virtual slides.

**Methods:** The participants (number limited to 8) access to the e-training workshop through a collaborative platform for image visualization and communication (Teleslide-TRIBVN) by the Website of the SFP. The minimal required equipment includes a 10 Mb/sec Internet connexion, a PC computer with Firefox or Chrome, and a microphone/speaker device. The virtual slide viewer (Calopix) is available freely from the platform. In 2012, the virtual slides were
shared through Calopix multiviewer and comments through Skype. In 2013, Espace Collaboration (Orange Business Services) allowed to share both applications and audiotransmissions by a phone bridge.

The workshop includes 90 virtual slides from 45 biopsies with clinical information. An evaluation form is filled by the attendees at the end of the teaching.

Results: The e-training is divided in 7 evening sessions: 1 Pretest, 5 thematic sessions, 1 Posttest of 2h-2h30 each one. Virtual slides are available on the platform one week before the beginning and 4 weeks after the end of the teaching. Every case is associated with an on-line vote. Pretest and thematic sessions take place over 2 weeks and the post-test session 2 or 3 weeks later. A supplementary session on liver transplant pathology has been organized in 2013 according to the wishes of the attendees. During the sessions, the webconferencing allows the teachers to give the control of Calopix viewer to each attendee for analyzing and discussing the cases in a collective manner. Each thematic session is concluded by a slide show commented by the teachers.

The fluidity for screening virtual slides is correct with hospital Internet access but not yet optimal with personal Internet access. The interactive sessions require hospital Internet connexion for the teachers to share the viewer application with the attendees. Nevertheless the participants greatly appreciate the flexibility and more than everything the interactivity of the teaching.

Conclusion: This experience of e-learning in pathology is positive in spite of some technical weaknesses which still need improvements. Short e-learning of 3 hours on hot topics (ex new tumor classification) will be soon proposed by SFP to test a new e-training format.

Potential of vibrational imaging in nephrology: detection of HydroxyEthyl Starch (HES) in renal tubules by Raman microspectroscopy in third generation HES – associated osmotic nephrosis lesions
Nicolas Elie, Lori Bridal, Olivier Piot
FRANCE

HydroxyEthyl Starch (HES) has been one of the most commonly used colloid volume expanders in intensive care units for over 50 years. First and second generation HES, with a high molecular weight (≥200 kD) and a high degree of substitution (≥ 0.5), has been associated with both renal dysfunction and osmotic nephrosis-like lesions on histological studies. Recently, third generation HES (130 kD/ < 0.5) has also been shown to impair renal function in critically ill adult patients. One hypothesis to explain the renal toxicity of HES is the accumulation of macromolecules in renal tubular cells, where they cannot be degraded because of their physicochemical properties. However, tubular accumulation of HES has never been demonstrated in the human kidney. This study reports four cases of biopsy-proven osmotic nephrosis-like lesions associated with impaired renal function related to third generation HES administration. In addition, this study describes a new method to determine and image the presence of HES in tubular sections using Raman microspectroscopy. Raman spectral “fingerprints” were defined for HES and these were observed on renal biopsies using Raman microspectroscopy. Raman spectral images of kidney biopsies from patients with HES-associated osmotic nephrosis and ten negative controls (HES-free tissue) were compared. The percentage area with positive HES-specific signals was significantly higher in cases than in negative controls (23.48% ± 28 vs 0.87% ± 1.2; p=0.004). In conclusion, this study shows that renal impairment related to third generation HES administration is associated with osmotic nephrosis-like lesions and HES accumulation in the kidney.

Automatic spectral histology of human colon tissues by infrared microimaging and cluster validity indices
Thi Nguyet Que Nguyen, Pierre Jeannesson, Audrey Groh, Dominique Guenot, Cyril Gobinet
FRANCE

Introduction: Fourier Transform Infrared (FTIR) spectral microimaging is an efficient label-free optical method to spectrally and spatially analyze the biochemical composition of biological samples. Recent studies have shown its potential to realize a real spectral histology when applied on human tissue sections and associated to clustering methods such as K-Means (KM). A limiting factor of the previously published studies is the empirical choice of the number of clusters.

Material and Methods: Ten formalin-fixed paraffin-embedded tissue blocks of normal zones were prepared from colon parts surgically removed from patients with colon cancer. For each block, two consecutive 6µm thick slices were
cut with a microtome. The first slice was mounted on a calcium fluoride (CaF₂) window for FTIR image acquisition performed with a Spectrum Spotlight 300 FTIR imaging system. The second slice was mounted on a glass window and stained by Harris’ Hematoxylin and Eosin (HE) for conventional histology analysis by an experimented pathologist. The acquired spectral images are then submitted to KM clustering with a number of clusters varying between 2 and 20. To realize an objective and automatic spectral histology, a new hierarchical double application of cluster validity indices (CVIs) to KM results is proposed in this study. This new methodology was tested using nine classical CVIs: Dunn, DB, Silhouette, XB, PBM, Sym-Index, COP, SV and OS.

Results and discussion: The classical application of CVIs on the FTIR images failed to retrieve the principal structures of human normal colon by underestimating or considerably overestimating the real number of clusters. CVIs are known to work correctly for dataset composed of compact and separated clusters. However, clusters generated by infrared data of biological samples do not fulfill these hypotheses. The proposed hierarchical double application of PBM and Sym-Index succeeds in retrieving the principal structures of human normal colon, namely the crypts, the lamina propria mucosae, the lamina muscularis mucosae and the submucosa. This new procedure thus permits to realize an automatic spectral histology of human normal colon.

The suitability of hierarchical application of CVIs to FTIR images is yet not clear. We suppose that the FTIR data have a hierarchical structure, i.e. few main clusters are composed of nearby and overlapped sub-clusters. However, this hypothesis needs to be confirmed. Furthermore, we proposed a two-layers CVI application, while the number of layers is obviously dependent on the considered dataset. An objective criterion needs thus to be defined to automatically estimate the number of required CVI application layers.

Conclusion: Spectral histology is a modern concept associating FTIR imaging with clustering. To overcome the manual and thus subjective choice of the number of clusters, we proposed in this work a solution based on a hierarchical application of CVIs on KM estimated partitions. Applied on FTIR images acquired on human normal colon slices, this procedure using PBM and Sym-Index CVIs achieves an automatic spectral histology since all the tissue structures are estimated.

The influence of the microscope lamp filament colour temperature on the process of digital images of histological slides acquisition standardization

Anna Korzynska, Łukasz Roszkowiak, Dorota Pijanowska, Wojciech Kozłowski, Tomasz Markiewicz

POLAND

Introduction: the aim of this study is to compare the digital images of the tissue biopsy captured with optical microscope using bright field technique under various light conditions. The range of colours variation in immunohistochemically stained with 3,3'-Diaminobenzidine and Haematoxylin tissue samples is immense. One of the main sources of colour variation in image is inadequate setting of camera's white balance to microscope's light colour temperature. The examination of the dependence of colour variation from microscope's light temperature and settings of camera is done as an introductory research to the process of automatic colour standardization. The results analysis of statistical image descriptors (range, mean and median of chromaticity on a and b channels from CieLab colour space and luminance L) averaged for images acquired in the same light conditions and camera setting leads to the following

Conclusion: (1) the images collected with white balance setting adjusted to light colour temperature clusters in cretin place chromatic space, (2) the process of white balance correction for images collected with white balance camera settings not matched to light temperature moves image descriptors into proper chromatic space but is paid by luminance affection. So the process of the image unification in a sense of colour fidelity can be solved in separate introductory stage before the automatic image analysis.
Parallel computing in image analysis using BrainVISA software: application to histopathological staining segmentation in whole slide images

Yaël Balbastre, Nicolas Souedet, Denis Rivière, Yann Cointepas, Michel Vandenberghe, Anne-Sophie Hérard, Thierry Delzescaux
FRANCE

Introduction: The development of whole slide imaging (WSI) techniques offers tremendous potential applications in preclinical research, through the digitization of entire tissue sections at the cellular resolution. We have been working on brain wide image analysis at the mesoscopic scale (resolution of a few micrometres) for several years and scaling up to the sub-cellular level opens the way to accurate quantification (shape, density) of histopathological and cellular markers. However, the transfer of mesoscopic processing techniques to whole slide microscopic images raises several issues. The treatment of tens of millions of pixels weighting several gigabytes is heavy in terms of both memory and computation. A satisfying way to reduce computation time is to parallelize the processing at the image (for large datasets) or sub-image (for large images) level.

Materials and Methods: We developed a new input/output library for BrainVISA/Anatomist (www.brainvisa.info), a freely available software dedicated to neurological image visualization and processing (T1 MRI toolbox, BrainRAT histology toolbox). This library allows partial reading and writing of in-house image format as well as common virtual microscopy formats using OpenSlide (www.openslide.org). This system was linked to a colour-based machine learning algorithm to segment entire histological sections of a mouse brain digitized using Leica SCN400F whole slide scanner at 0.25 µm in-plane resolution. Brain sections were virtually divided into sub-images whose processing was distributed on a computing cluster (64 3GHz processors) using SomaWorkflow (www.brainvisa.info/soma-workflow/) – a unified and simple interface to parallel computing resources.

Results and Discussion: Several mouse brain sections with various stainings (Thionine, Iba1, CD68) were successfully processed. In the case of an Iba1-stained section image of 82 million pixels, microglia segmentation takes 3 hours with one core versus 13 minutes with 16 cores (speedup: 14) and 5 minutes with 64 cores (speedup: 36). It is noteworthy that parallelization not only fastens image processing but also lightens the memory charge on each local computing resource in the same proportion. This is especially convenient when processing big images on a loosely connected computer cluster. However, memory resources are not automatically managed by SomaWorkflow and users need to predict memory usage and choose their strategy accordingly. The parallelized version of our algorithm is highly scalable since feature vectors are computed on small neighbourhoods around each pixel and classified independently. Thus, up to 97% of the classification algorithm is parallelized.

Conclusion: Upgrading image processing algorithms dedicated to mesoscopic whole slide imaging or conventional microscopic imaging towards whole slide microscopic imaging is made possible by our developments. The first results obtained in colour segmentation of entire brain sections are promising. This work opens the way to potential high throughput processing of whole slide images as well as 3D reconstruction and analysis of WSI data using BrainRAT toolbox.

Digistain: A Digital Staining Instrument for Histopathology

Hemmel Amrania, Laurence Drummond, Chris Phillips, Laura Woodley, Sami Shousha, Charles Coombes
UNITED KINGDOM

Introduction: To report a new technique for providing a quantitative measure of breast tumour grade in breast core biopsies.

Method: Digistain images are derived from the relative concentrations of the amide (cytoplasmic) and phosphate (nuclear) moieties present in tumour cells. Reflecting the Nuclear/Cytoplasmic ratio in tumour cells, they allow these respective concentrations to be evaluated as a measure of malignancy. A suitably calibrated combination of these concentrations is used to generate false colour computer images that reproduce not only tissue morphology, but also accurate and quantitative maps of chemical composition throughout the tissue section. Unlike other digital pathology tools employed to assist diagnosis, Digistain uses a unique optical signature to analyse the chemical make-up of a biopsy quantitatively, using unstained sections. The technique is unaffected by the subjectivity of grading, particularly grade 2 and intermediate cases. Within minutes of loading a slide it yields a reproducible and numerical grading score that
helps physicians and patients decide on the most effective treatment plan.

**Results:** 20 breast cancer core biopsies were classified according to their respective grades. The Digistain score of each group was found to correlate strongly across the grades, thus validating the use of this index as a suitable indicator of malignancy.

**Conclusion:** We believe the new Digistain approach provides for the first time a cost effective and quantitative measure of tumour grade. This can be developed to deliver an effective assessment of prognosis and recurrence risk beyond traditional qualitative measures based on H & E staining protocols.

**Diagnostic question & answer thanks to Molecular Signature Detection with Multi-Modal Microscopy Scanner - M3S EU project**

Jacques Klossa, Maurice Bowe, Alexandre Cauchon, Bernard Chatelain, Alexandre Civet, Edouard Cornet, Boris Ecker, Cyril Gabinet, Michel Manfait, Dominique Mazier, Olivier Piot, Philippe Rideau, Alexandre Templier, Marc Thellier, Xavier Troussard

BELGIUM, FRANCE, GERMANY, UNITED KINGDOM

For many diseases, diagnosis through morphological studies at microscopic scale is the first step conducting to deeper investigations. Yet current tools barely benefit from new biophotonics technologies that could facilitate and greatly improve both early diagnosis and referral to personalized treatments. Associated to an automation of data collection and analysis, such solutions could constitute powerful instruments for better care of patients.

M3S thus intends to gather complementary options developed independently by partners and now reaching the maturity to be integrated and evaluated in routine care clinical settings. Once combined, they will efficiently answer the clinical needs and provide a more comprehensive alternative to existing approaches.

The final global platform will include three major components: i) an innovative biophotonics-based imaging instrument associating a Raman micro-spectrometer and an accurate stage controller to scan unstained samples and acquire spectral signatures of representative cell or cell compartment; ii) a powerful image workstation localizing and identifying objects of interest and capturing exploitable data; iii) a new and efficient data extractor and analyzer based on the QFinder® algorithm detecting and characterizing molecular signatures through exhaustive exploration of multivariate data.

The approach aims to i) limit the use of markers and dyes whose preparation is difficult to standardize, ii) identify new intrinsic physico-chemical markers specific for a given pathology and iii) detect local configurations where risks or opportunities have shown to be significantly higher or lower than average, allowing a more sensitive diagnosis and a more secure and adapted selection of treatments.

Although the developed technologies can be applied in various situations, the efficiency of the integrated solution will be tested in two pathologies for which previous information have been collected: Chronic Lymphocytic Leukaemia and Malaria.

The 9 involved partners are: Medyc unit at URCA University; POLYTEC, optical and biophotonic measurement systems; Objective Imaging, control of microscope automation; Quinten, biomedical data for biotechnology; Nikon France, photonic microscopes and imaging systems; TRIBVN, diagnostic microscopy imaging, Parasitology/Mycology department at Pierer et Marie Curie University; Cyto-haematology department at Université Catholique de Louvain, Cyto-haematology department at Caen University Hospital.

The M3S project is co-funded by the the EU CIP ICT PSP programme.

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**PechaKucha Session**

*chaired by Thomas Schrader*

**Rapid diagnosis of Wilson disease on tissue sections or biopsies using X-ray fluorescence**  
*Slavka Kascakova*  
France

**AIDPATH: Academia and Industry Collaboration for Digital Pathology - FP7-EC**  
*Gloria Bueno*  
Spain

**The fastest way to have a look of previous archived biopsies**  
*Luca Molinaro*  
Italy

**Morphological investigation of elastography signal on breast cancer using virtual slide superimposition**  
*Myriam Oger*  
France

**Pecha kucha, scientific slam & other tools for a agile scientific culture in digital pathology**  
*Thomas Schrader*  
Germany

Note: It is possible to register new pecha kucha presentations until the beginning of each session.
ADCIS: A set of advanced tools to analyze virtual slides
Bruno Laÿ and Gervais Gauthier
FRANCE

Since its inception in 1995, ADCIS has been developing very advanced tools in the fields of Image Processing/Analysis including its flagship product Aphelion Imaging Software Suite. Since a few years, ADCIS has been receiving requests for adding the capability to process and display very large images including pathology images. It was then decided to address this issue by developing specific tools and adapting the software to new 64 bit PC architectures that let the user display and process large data from an intuitive user interface. New tools include the implementation of a multi-resolution approach to dramatically improve the processing speed of large images, and the focus on biological applications in partnership with the research team PATHIMAGE of the Centre François Baclesse and Caen University. Below is a list of software products targeted to pathological applications:

- **Aphelion Dev**: The Developer software environment to prototype and deploy advanced applications supporting regular 2D images, 3D images, and virtual slides. The support of tiled images has been added to let the user display and process virtual slides using the large set of image processing operators available in Aphelion Dev. Software capabilities include the automatic extraction of areas of interest, cell detection, measurement extraction, and analysis report generation. Aphelion can be used to develop applications in most of the common fields.

- **Classifier Builder**: A specific tool including a very comprehensive environment to classify cells based on a set of descriptors. It is based on an application front-end to define the classifier derived from a set of training data that have been sorted by experts into categories. Three classifiers are currently supported in the software: Fuzzy Logic, Neural Network, and Random Forest. Once classifier parameters are defined, cells can be automatically classified into categories.

- **Comet Assay**: A specific extension to automatically process images of Single Cell Gel Electrophoresis (SCGE). This test is used in oncology to predict the intrinsic radio-sensitivity of tumors, or to study the individual sensitivity of genotoxic agents.

- **Ploidics**: A software system to automatically measure abnormal DNA content in individual cells of a mixed cell population from digital micrographs of cytopathology specimens.

- **Stereology Analyzer**: A software application to analyze digital images of histology specimens using the long-standing and accepted stereology technique. This statistical technique easily allows achieving the best estimate of biomarker parameters in user-defined regions of interest.

- **Virtual Image Capture (VIC)**: A software application to control an optical or scanning electron microscope system to automatically capture images of the whole slide or a user-defined area at a given magnification.

- **Virtual Image Stitcher (VIS)**: A software application to automatically stitch images captured using VIC, generate a digital virtual slide, and visualize it at any given magnification. There is no limit on the virtual slide size thanks to the unique and innovative stitching technique available in VIS.

Application examples based on Aphelion software tools will be presented during the conference: Analysis of a virtual slide of breast cancer, and analysis of functional areas of an oyster.

**CLOUD PATHOLOGY: A New Massive CPG Test: Managing a Digital Pathology Start Up**
Filippo Crivelli, Giampiero Duglio, Stefano Pezzati, Anna Bauer
ITALY

Following the CPG report on a Preliminary Slide Scanner Throughput Evaluation in an Intensive Digitalization Facility Setting at the 11th Congress in Venice 2012 (Diagnostic Pathology 2013,8, Suppl. 1), the Paris presentation is centered on the first results of a new CPG research activity finalized to evaluate the effects of a massive use of the Digital Pathology in a Multi-hospital Pathology Department, throughout an outsourcing agreement.

The presentation starts by describing the main characteristics of the Busto Arsizio Group Hospitals, the protocol of the experimental activities and the technological choices (starting from the Hamamatsu Nanozoomer XR). Then the main issues regarding the laboratory activities and the pathologists’ diagnostic procedures. At the end, the present conclusions and results of this experiment will be explained.

The results on the massive use of the Digital Pathology in the surgical pathology of Linköping Hospitals (Sweden), very similar to the Italian CPG experiment, will suggest the availability of a European standard approach to Digital Pathology.
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ANDRAL: Open Access Solution of Tele-Expertise in Cytology

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Diagnostic Challenges and Advantages of International Telepathology between Two Medical Institutions

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## A7: MODELS, DATA MINING AND KNOWLEDGE FORMALIZATION IN PATHOLOGY

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## B1: IMAGE ANALYSIS-1

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Multiple immunohistochemistry markers by digital image analysis reveal complex interdependencies but do not provide prognostic value in multivariate analysis models

**B5: QUALITY-2: QUALITY CONTROL**

Digital immunohistochemistry wizard: image analysis-assisted stereology tool to produce reference data set for calibration and quality control
Accuracy of an Automated Vessel Counting Algorithm in Four Different Tumor Types
Computer-aided her2/neu evaluation in external quality control (EQA) of breast cancer screening programme
Comparative Study between Quantitative Digital Image Analysis and Fluorescence In-Situ Hybridization (FISH) of Breast Cancer Equivocal HER2 Score 2+ cases
FISHQuant quantification algorithm validation in the clinical molecular diagnostics

**B6: COMPUTER AIDED DIAGNOSIS SYSTEM**

Frequential versus Spatial Colour Textons for Breast TMA Classification
Computer-aided diagnosis from weak supervision: A benchmarking study
Automated identification of cell nuclei in tissue sections
Application of Computerized Digital Image Cytometry of DNA Aneuploidy for Cervical Cancer Screening in China
Introduction of a cancer tissue detection method via homology

**B7: TECHNOLOGY ADVANCES-2**

Quantitative assessment of liver steatosis using infrared microspectroscopy
Performance of full-field optical coherence tomography (FFOCT) digital imaging for prostate cancer diagnosis
The FourierScope - a New Whole Slide Imaging System Based on Fourier Ptychographic Microscopy
Dry mass and cell cycle follow-up from Quantitative Phase Imaging
Tissue imaging with quantitative phase imaging (QPI)

**POSTERS - TELEPATHOLOGY, VIRTUAL MICROSCOPY, IMAGE ANALYSIS, IT IN PATHOLOGY**

From Microscopy, Imaging to Clinical Research: A Latin American Perspective
Ki67/KL1 immunohistochemical double stains increase accuracy of Ki67 indices in breast cancer and simplify automated image analysis
On-Demand Model for Digital Pathology
Digital pathology: a new tool in Pathology department
Multimodal biomarker study by PET and digital microscopy of the response to sunitinib on a luminal B-type mammary carcinoma model
Acceptance of digital tumor board presentations in two medical institutions
Virtual slides versus binocular microscope use. An orthoptic evaluation of visual strain
FlexMlm: towards efficient/effective Collaborative Digital Pathology
The TASTE* (Telepathological Assessment of Histopathological and Cytological Techniques) Project: Aiming to define European pathology slide technical standards
Automated image analysis is superior to manual reading of HER2 expression in breast cancer
A segmentation method for images with subjective contours applied to immunohistochemistry-stained cell membranes
Comparison study between TIFF and downsampled images. Automated evaluation of cytokeratin-19 immunostained scanned breast cancer tissue microarray.
Telepathology network in Ile de France: a 18-month experiment project for frozen sections (telediagnosis) and second opinions (teleexpertise).
Image analysis in virtual slides: Comparison between the expression of hormonal receptors and DNA ploidy (static cytometry) in breast carcinoma.
A Semantic Interoperability Framework for Facilitating Telepathology
WSI virtualization: value added representation of Whole Slide Images
Analyzing huge pathology images with open source software with an application to gliomas
Integration tools of the digital pathology system into the research biobank management solution
POSTERS – TECHNOL. ADVANCES, E-LEARNING, QUALITY, COMP. AIRED DIAGNOSIS

Infrared spectral imaging to automatic assessment of tumor response
3D quantitative histopathology in the mouse brain: from mesoscopic to microscopic scale
HER2 immunohistochemical assessment of breast carcinoma by image analysis in five vs. ten fields of view and its correlation with fluorescence in situ hybridization.
Typing less common ovarian tumors: A training tool based on a pattern-based algorithm applied to a set of 20 virtual slides.
Virtual Slides for Teaching Pathology & Hematology
Computer-assisted diagnosis of malaria infection with an automated microscopy system
Full-field optical coherence tomography (FFOCT) of breast tissue: a new diagnostic tool for evaluation of breast proliferations?
Evaluation of classical and virtual slide teaching methods of practical histology
Discrepancies between diagnoses of real and virtual microscopy compared with intra and inter-observer’s variation
Teaching Anatomical Pathology at the University of Barcelona: Transition to Virtual Slides and Virtual Microscopes
Completely automated integrated system for prostate cancer grading
Using a rich internet application to teach histology
Virtual slides for continuing medical education in pathology
Potential of vibrational imaging in nephrology: detection of HydroxyEthyl Starch (HES) in renal tubules by Raman microspectroscopy in third generation HES –associated osmotic nephrosis lesions
Automatic spectral histology of human colon tissues by infrared microimaging and cluster validity indices
The influence of the microscope lamp filament colour temperature on the process of digital images of histological slides acquisition standardization
Parallel computing in image analysis using BrainVISA software: application to histopathological staining segmentation in whole slide images
Digistain: A Digital Staining Instrument for Histopathology
Diagnostic question & answer thanks to Molecular Signature Detection with Multi-Modal Microscopy Scanner

PECHAKUCHA SESSION

Rapid diagnosis of Wilson disease on tissue sections or biopsies using X-ray fluorescence
AIDPATH: Academia and Industry Collaboration for Digital Pathology - FP7-EC
The fastest way to have a look of previous archived biopsies
Morphological investigation of elastography signal on breast cancer using virtual slide
Pecha kucha, scientific slam & other tools for a agile scientific culture in digital pathology

INDUSTRY SYMPOSIUM

ADCIS: A set of advanced tools to analyze virtual slides
CLOUD PATHOLOGY: A New Massive CPG Test: Managing a Digital Pathology Start Up
with resources from MICO French ANR TECSAN project and with the kind support of: