Common triggers of HLH in adults		n	%	Frequency of HLH diagnostic criteria (must meet 5 of 8)	n[2]	%
Infectious	Acute EBV	7	19.4	Fever	30/31	96.8
Subtototal n=18 (50%)	Bacteria [1]	5	13.9	Splenomegaly	16/20	80.0
	Histoplasmosis	4	11.1	Cytopenias of at least 2 lineages	29/35	82.9
	Mycobacterium kansasii	1	2.8	Fibrinogen (<150 mg/dL)	15/36	41.7
	CMV	1	2.8	Triglycerides (>265mg/dL)	15/31	48.4
Malignancy	T cell Lymphomas	5	13.9	Ferritin>500 mcg/L	35/35	100
				Ferritin>10000 [3]	23/35	65.7
Unclear		13	36.1	sIL-2r	4 of 6	66.7
Total		36	100	NK function [4]	2 of 3	66.7

Most frequent underlying conditions				1. *Burkholderia sp, Rothia mucilaginosa, Stenotrophomonas*, others
Crohn's	3	CHL	2	2. Denominator is variable: data not available
Autoimmune	3	MDS	4	3. Reported as more specific in children
Hemodialysis	2	Healthy	6	4. Measured by the 51-Cr release assay
NA	16	Total	36	

Conclusions: This is the largest series of HLH cases in adults. Acute EBV and bacterial infections followed by histoplasmosis were the most frequent diseases identified in this series. Neoplasms and autoimmune diseases accounted for a minority of cases. The institution of specific immunosuppressive therapy did not change mortality rates, unlike what has been demonstrated in studies of the pediatric population.

1566 Extra-Oral Plasmablastic Lymphomas as a Predominant Involvement Pattern in AIDS Patients of an Inner City Hospital

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Background: Plasmablastic lymphoma (PBL) is defined as a rare aggressive lymphoma characterized by a diffuse proliferation of large neoplastic B-cells with predominance of immunoblastic/plasmablastic morphologic features and a plasma cell –like immunophenotype.Originally, PBL was outlined as an aggressive, B-cell lymphoma, primarily occurring in the oral cavity and most frequently arising in association with human immunodeficiency virus (HIV) infection.

Design: From 2004 to 2012, 14 cases of patients diagnosed with PBL were identified. Electronic medical records were used for extraction of demographic data, HIV status, HIV-associated risk factors, laboratory data, PET imaging scan results, pathology reports and flow cytometry reports. Pathology slides with immunohistochemistry stains were reviewed.

Results: Of the 14 patients with PBL, there were 13 men and one woman with an age range of 26-63 years. 13 patients were HIV + (92.9%), and one patient was HIV -. Less than one fourth (21.4%) of patients (3) presented with oral lesions and the remaining 71.5% presented with extra oral disease including anus, colon, rectum (5), stomach (2), liver (1), penis (1), inguinal node (1) and lungs (1). Most of the HIV + patients were on HAART therapy (84.6 %) treatment and one patient was reported as non-compliant. In the large majority of cases, PET scan demonstrated disease involving other organs, with only one patient free of widespread lymphoma. The bone marrow was infiltrated in 6 cases (42.8%). The most common HIVassociated risk factor was men having sex with men (MSM) (42.8%). The single HIV negative patient presented with disease in the oral cavity/gingival, and was the only patient noted to be in remission from the disease.

Conclusions: Contrary to other studies, our series demonstrates that the majority of PBL in HIV positive patients occurred primarily in extra oral and extra nodal sites. The most common extra oral sites were the ano/rectal area followed by the stomach, with single occurrences of cases in the lungs, liver and penis. Most of the HIV positive patients were on HAART therapy, with disease involving other organs and bone marrow. Diagnosis of PBL is challenging, particularly when it arises in unusual extra oral locations and present as an undifferentiated neoplasm. In those cases an appropriate immunohistochemical pattern including plasma cell markers and B-cell markers should help in diagnosis of such tumors.

1567 Non-Classical MHC-1 Class 1b HLA-E Expression Levels Are Maintained in HIV-1 Infected CD4 Positive T Cells

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Background: HLA-E is a non-classical major histocompatibility complex (MHC) class 1b molecule that is expressed on virtually all cells. Binding of the HLA-E ligand to the natural killer (NK) cell inhibitory receptor CD94/NKG2A is sufficient to protect cells from NK cell mediated lysis. Infection of CD4 positive T lymphocytes with HIV-1 leads to significant downregulation of CD4 and classical MHC class 1a HLA-A and HLA-B expression. Although a few studies have reported increased HLA-E expression with HIV-1 infection, this effect is not well characterized. In this study, we sought to determine the effects of HIV-1 infection on HLA-E expression levels in CD4 positive T cells.

Design: Stimulated peripheral blood mononuclear cells from healthy volunteer donors were depleted of CD8 positive T cells and infected with the NL4-3 HIV-1 virus strain at varying multiplicities of infection. Cells were harvested at 2 days, 4 days, 7 days, and 10 days, and flow cytometry was utilized to determine expression levels of CD3, CD4, HIV-1 p24, HLA-A,B,C, and HLA-E. Expression of HLA-E in infected p24 positive cells was compared to levels in uninfected p24 negative cells at each timepoint.

Results: Following infection with HIV-1, significant p24 expression was identified in a subpopulation of CD3 positive T cells with low or negative expression of CD4 (mean peak infection of 9.5% of total T lymphocytes). This subpopulation of p24 positive T cells exhibited persistent downregulation of MHC-1 HLA-A and B beginning at the day 4 timepoint and continuing throughout the remainder of the experiment, relative to the p24 negative T cells (mean 72% downregulation). However, no persistent enhancement or downregulation of HLA-E expression was observed in p24 positive T cells compared to p24 negative T cells. HLA-E expression levels remained constant in HIV-1 infected CD4 positive T cells in contrast to the markedly decreased expression of HLA-A and B.

Conclusions: Review of the literature shows that various studies have come to different conclusions as to whether HLA-E expression is actively enhanced or remains stable in HIV-1 infected cells. In these experiments, our data suggest that HLA-E expression remains stable in CD4 positive T cells following infection with HIV-1 while HLA-A and B expression is markedly decreased. This selective downregulation of the classical MHC-1 class 1a molecules with maintenance of HLA-E expression allows infected cells to escape NK cell mediated lysis. Further studies seek to identify potential viral products that may be involved in the relative stabilization of HLA-E expression.

Informatics

1568 Synoptic Report Generators for Molecular and Ancillary Test Reporting in Anatomic Pathology Laboratory Information System (APLIS)

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Background: Synoptic reporting for cancer has increased the consistency, completeness of reporting, ease of interpretation and enabled searchable results. Many of these are done using embedded synoptic report generators that allow for check box or short field reporting. We sought to develop a similar system for reporting of molecular tests and ancillary tests using a custom-built embedded synoptic report generator in our anatomic pathology laboratory information system(APLIS) (PowerPath™, SunQuest).

Design: We built and implemented custom synoptic reporting- based embedded templates using the worksheet function within our APLIS (PowerPath™) for the different molecular and specialized ancillary tests. Molecular tests included a comprehensive lung cancer (EGFR, ALK, KRAS, BRAF), colon carcinoma reporting templates (KRAS, Microsatellite instability (MSI), BRAF, MSI immunohistochemistry) and T- and B- cell clonality. Ancillary tests included breast carcinoma comprehensive reporting template (ER, PR, HER2 IHC, and HER2 FISH) and UroVysion™ reporting templates.

Results: The embedded worksheet reports had the following advantages of standard reporting: 1) Ability to generate a summary results section at the top of report; 2) ease of reporting with standardization of the reporting; 3) ability to generator specific comments specific to prognosis and implications for therapy for a particular mutation (EGFR, KRAS mutation); 3) generation of quality assurance reports such as rates of positivity for mutations, rate of ER/PR/HER expression relative to degree of differentiation of tumor, correlation of cytology with UroVysion™ findings; 4) creation of a searchable database for items such as all particular mutations and ER/PR/HER2 expression; 5) ability to incorporate free text comment sections for needed flexibility.

Conclusions: Embedded synoptic report generators for molecular and ancillary reporting within an APLIS has improved consistency, accuracy, ease of reporting and interpretation and allowed for easier quality assurance tracking and research applications.

1569 Implementation of a Pathology Teaching Website with Integrated Whole Slide Imaging for Greater Compatibility with Tablets/Smartphones

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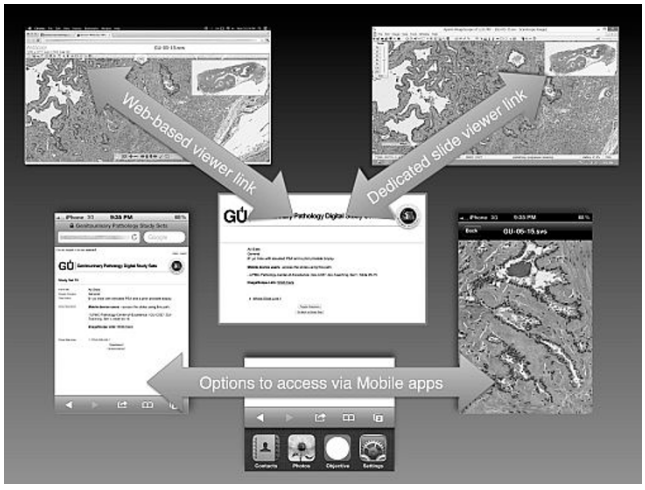
Background: Study sets involving digitized virtual slides at our institution had limited viewer flexibility and accessibility, typically requiring either a flash-based web browser or a dedicated slide viewing application. We modified our online Genitourinary Pathology Study Set, complete with digital whole slide imaging, to be viewable on computers and smartphones/tablets with integrated toggles of answers and explanations, while maintaining compatibility with different slide viewing programs according to the user's preference.

Design: Website elements, originally coded in HTML, were simplified to enhance readability on mobile devices while retaining password-protected access. Hyperlinks to virtual slides were modified for slides to automatically open using an installed slide viewer, instead of a new web browser window. Multiple links to each slide were created to provide compatibility with web-based viewers, Aperio ImageScope for computer users and several tablet/phone slide viewers including Aperio ePathViewer, Objective or Wholeslide.



Results: 4 of 32 pathology residents accessed the remodeled website using primarily desktops or laptops, 10 of 32 used primarily smartphones or tablets, and 18 of 32 alternated between devices with desktop and mobile operating systems. All 32 users reported improved navigation and self-assessment capabilities, because they accessed slides using their preferred slide viewing application; they were not restricted to using one program or a Flash-based web browser. Users also reported improved methods of studying under the new website, such as reading textbooks about the entities they studied on the same device used to access the slides.

Conclusions: Virtual slide sets with multiple-device compatibility allow users to remotely access data via the time, place and 'gadget' of choice, thereby facilitating access to digital slides while reducing the limitations of previously used methods. All of our urologic pathology study slides are accessible using smartphones/tablets. In addition, our model was expanded for users to create post-rotation exams, which residents may take at their preferred time and place; such methods could be standardized for competency assessment in other anatomic pathology rotations.

1570 Standardized Checklist/Synoptic Tool for Reporting of Uropathology Neoplasms: Implementation and Experience with 6885 Resected Specimens

W Anani, A Piccoli, L Pantanowitz, A Parwani. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Cancer checklists comprising of standardized data elements are valuable tools that clinicians use to guide them in managing patients. We describe our experience with the use of an electronic synoptic worksheet entry tool within our existing workflow for reporting thousands of malignant genitourinary resections.

Design: We used a synoptic reporting tool as part of an existing laboratory information system (LIS), CoPathPlus (Cerner). We modified the CAP checklists into worksheets for genitourinary malignancies. These synoptics have been in use since 2003 in our LIS and have been modified on multiple occasions to incorporate changes in The College of American Pathology checklists (www.CAP.org) or AJCC TNM staging protocols. Data entered into the worksheets are present in reports as discrete data elements with categories such as tumor site and size, histologic type, grade, perineural invasion, angiolymphatic invasion, margins, and TNM staging.

Results: A total of 6885 genitourinary specimens had synoptic reports, including in-house and consultation cases. Separate templates for prostate (4398), kidney (1862), renal pelvis (239), testis (229), ureter (116), penis (21), urinary bladder (8), urethra (7), and resection for Wilms tumor (5) have been created. The synoptic templates can be attached to a specimen at any point in the workflow before completion of the case with a greater than 95% compliance rate. Minimal technical support is needed to maintain the program and accuracy of the checklists.

Number of Worksheets Completed By Organ System

Organ System		Number of Synoptics Completed	Data Elements
Prostate		4398	112
Kidney			
	Kidney	1862	86
	Renal Pelvis	239	88
	Resection for Wilms Tumor	5	88
Testis		229	88
Ureter		116	84
Penis		21	59
Urinary Bladder		8	106
Urethra		7	123

Conclusions: Use of an electronic synoptic report within the existing LIS is recommended because of the benefits it provides to its users. The intuitive interface and streamlined format allows a user of any experience level to input information into standardized worksheets. This is essential to maintaining the efficiency of workflow in high volume centers such as ours. Infrequent updates and program maintenance minimizes interruptions and provides a reliable tool in creating synoptic reports. The data collected represents a rich source of highly annotated biospecimens that can now be harnessed for clinical outcomes, cancer registry, research and patient safety databases.

1571 Telepathology for Frozen Section Analysis: A Validation of Remote Meeting Technologies (RMT)™ Software

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Background: The University of Wisconsin Hospital and Clinics is supporting frozen section(FS) capability at a partner institution over 40 miles away. The Department of Pathology was tasked with finding the most cost effective, accurate, and efficient solution to providing this service. After review of many techniques, including virtual scanning, real time imaging was chosen. To prove this method was just as effective as direct microscopy, a validation/correlation study was performed. This also functioned as a training/competency assessment for participating pathologists allowing them to become familiar with making diagnoses from digital images.

Design: Six pathologists were given a set of 20 FS slides and asked to interpret them via our chosen telepathology system, Remote Meeting Technologies (RMT)™. The slides were chosen after a review of common FS slides seen in the partner institution. The more difficult cases were favored to show that the RMT system could accurately display diagnostic features in a digitized image. After 3 months or more of lag time, the slides were reordered and presented to the same pathologists for direct microscopy examination. The answers from each exam were then correlated to determine if any difference existed between the two methods of diagnosis.

Results: The Cochran-Mantel-Haenszel (CMH) test found outstanding correlation between telepathology and direct microscopy with a p value of <0.0001. The null hypothesis in this analysis was that there was no correlation between the two methods. The exceedingly low p value gives extremely strong evidence against that null hypothesis.

McNemar's test for each pathologist

Pathologist	P value from McNemar's test	Comment
D	1	Telepathology and microscopy agree
F	.083	Telepathology and microscopy agree
G	.083	Telepathology and microscopy agree
H	1	Telepathology and microscopy agree
I	.083	Telepathology and microscopy agree
J	.16	Telepathology and microscopy agree

Conclusions: The RMT system provides 1920 x 1080 pixels of resolution which is the equivalent to high definition. This provides the diagnosing pathologist with an exceptionally sharp view of diagnostic features. Our study shows that our telepathology system is valid for interpreting FS. The collected data provides excellent evidence of a high degree of correlation between the two methods. More studies of this type need to be performed on other systems being developed to prove their validity in FS diagnosis and the pathologists' competency in using them.

1572 Optical Coherence Tomography (OCT) for Intra-Operative Style Interpretation of Endometrium

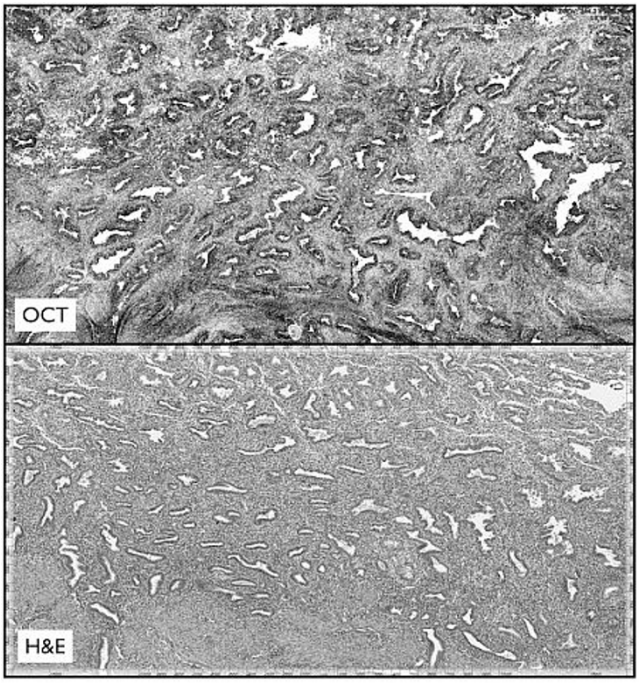
IC Cucoranu, JL Fine. UPMC, Pittsburgh, PA.

Background: Optical coherence tomography (OCT) offers direct microscopy of tissue without glass microscope slides. In addition to "room temperature" frozen sections or guided grossing, OCT is also being developed for in vivo imaging with pathological diagnosis. This is a pilot study using a new OCT system to look at endometrium, which is difficult to sample intra-operatively and could be amenable to in vivo imaging.

Design: Signed out, formalin fixed hysterectomy specimens were selected from 2 weekly signout rotations of one of the authors; cases were excluded if the specimen was unsuitable for post-signout imaging (e.g., morcellated uterus, endometrium totally submitted for sampling, etc.). 33 samples from 21 cases were imaged using OCT (Light CT, LLTech, Paris, France); the tissue was submitted for histology, H&E slides were prepared and digitized in whole slide image format (XT, Aperio, California, USA). Regions of interest from OCT and H&E were organized into two powerpoint presentations: training images (10 sets of matched OCT and H&E) and test images (32 sets of OCT only). Three expert pathologists (<5 years experience) reviewed the images on 24" computer monitors. Data captured included time estimates and frozen section style diagnoses (first line with benign/malignant/defer; second line with diagnosis). Data was correlated with H&E diagnoses from a fourth subspecialist pathologist. Inter-observer agreement was calculated by using Fleiss's Kappa statistics.

Results: Pathologists spent 5-10 minutes on training images and 30-60 minutes on test images. Mean deferral rate was 10.66% (range 8-12%); benign/malignant discrepancy rate was 10.44%; 7 cases were not used (no endometrium (4), OCT scan was "enface" (3)). The overall inter-observer agreement between the three subject pathologists was moderate (κ=0.4329).

Conclusions: OCT-novice expert pathologists were able to interpret endometrium without traditional glass microscope slides! In highest-quality images different patterns were plainly visible (e.g. adenocarcinoma, proliferative or secretory patterns (Figure)). Image quality improved with experience. Tissue fixation impaired enface scanning. Enface images may permit wider-area screening intra-operatively, potentially even in vivo, therefore studies with fresh tissue are important.



1573 Evaluation of a Web-Based Telepathology Consultation Service Platform

IC Cucoranu, SA Yousem, G Romero Lauro, W Cable, J McHugh, AV Parwani, L Pantanowitz. UPMC, Pittsburgh, PA.

Background: Telepathology facilitates distribution of professional expertise over broad geographic areas. Various modes of telepathology have been adopted at our institution for over a decade to support pathology services at local, national and international sites. These telepathology initiatives have evolved to begin leveraging whole slide imaging (WSI) technology and the Internet. We recently developed web-based telepathology consultation portals that facilitate WSI sharing by local and international clients for second opionion, in a secure environment. Our aim was to evaluate our experience with these portals.

Design: A general portal was developed for all clients to securely upload WSI and pertinent clinical information, for transmission to our pathology specialty consultants. It employs a vendor neutral viewer, allowing use of multiple WSI file types. Clients can login securely to check the status of their cases and to view or print reports. Workflow is handled by managers who triage requests, monitor cases, and maintain personnel data. A client-specific portal required customization for individual clients (Table 1). This setup allows consultants to view WSI hosted on remote servers at the client's location, via Internet streaming. Image files are organized by scanner's software and no storage or transmission of personal health information is encountered.

Results: Streamlined workflow and users training has ensured prompt turn-around time and buy-in by pathologists. Post-launch feedback has resulted in customization to incorporate transcription services, peer-to-peer review for consultants, provision for issuing addenda or amendments, and improved WSI viewing experience. During a 10-months period, 75 teleconsultation cases were signed out by our subspecialty pathologists on the client's specific portal. The average case turnaround time was 3.86 days. Delayed cases have been attributed mainly to network outage problems.

Conclusions: Both client and consultant users' satisfaction with the telepathology consultation portal is positive. This technology provides faster and more convenient access to subspecialty pathology consultants.

Table 1. Telepathology Portals

General Portal	Client-Specific Portal
Simple and cheaper	Configurable
Image transfer required	No need for image transfers
Supports multiple WSI vendor formats	Customized to run only the client's WSI format
WSI files organized by folders	Files organized by scanner software
Customers enter PHI into the portal tool	No transmission or storage of PHI
Built-in billing component	No billing tool (contractual)

1574 Customizing Anatomical Pathology Laboratory Information System (AP-LIS) Tools To Augment Laboratory Workflow, Reporting, and Patient Safety

IC Cucoranu, SA Yousem, A Piccoli, AV Parwani, L Pantanowitz. UPMC, Pittsburgh, PA.

Background: Most Anatomical Pathology (AP) laboratory operations rely heavily on their laboratory information system (LIS). Therefore it is not surprising that pathologists have looked to the AP-LIS to meet increasing demands to handle electronic transactions, to accommodate theranostics, implement barcoding and other quality controls to enhance patient safety. Our aim was to review the success of recent AP-LIS customizations performed at our institution to meet these needs.

Design: Our AP-LIS (CoPathPlus, Cerner, v3.3) generates over 7500 results weekly from over 200 users at 14 sites. We analyzed four key LIS customization tools (synoptic reporting, barcoding, pre-signout QA evaluation, theranostic summation) and their impact after implementation.

Results: We employed 65 templates for synoptic reporting that were used in 47,286 surgical pathology reports since implementation. They improved turnaround time, results standardization, and reduced transcription errors. Barcoding implemented at two facilities reduced slide mislabeling errors and helped drive workflow. An automated, pre-signout QA tool significantly decreased amended reports after implementation further preventing diagnostic errors. A comprehensive theranostic summary incorporating ancillary tests assists clinicians with patient clinical management.

Conclusions: Customization of our AP-LIS has permitted our laboratory to support evolving technologies including electronic reporting, asset tracking and molecular testing by incorporating them into daily operations. These efforts have improved and standardized our testing process, facilitated automation, as well as enhanced patient safety, QA measures, and clinical patient management. Future efforts will include further enhancements to improve workflow and reduce errors.

1575 Validation of MMP-14 Quantitative Staining Evaluation in Ovarian Cancer Using Image Analysis

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Background: Visual immunostaining quantification is laborious and subject to significant intra and inter-observer variability. Automated image analysis is growing as an interesting and more objective tool for biomarker studies, especially when using tissue microarrays.

Design: Immunohistochemistry for the collagenase MMP-14 (Pierce, Rockford, Illinois) was performed on tissue microarrays built from 236 ovarian carcinomas. Cytoplasmic immunostaining was scored visually by two observers using a five-tiered scale (0%, 1-20%, 21-40%, 41-60%, 61-80%, 81-100%) and positive staining was graded into three intensity classes. Immunostaining evaluation was then submitted to automated image analysis on a random core sample using the CaloPix™ software (developed by TRIBVN, distributed by Agfa HealthCare). A protocol aimed at segmenting the tissue samples to exclude the non-epithelial component was first developed followed by an automated analysis of the percentage of positive epithelial cells and a three-tiered intensity scale.

Results: Although ovarian carcinomas have a high level of tissue heterogeneity, automated tissue segmentation was adequate in 49 out of 58 cores. Partial manual tissue segmentation was needed on the other cases. Differences in percentages of positive immunostained cell between manually and automated segmented zones varied between 1.2 and 17 % (median 3.2%). Overall, the percentage of automated stained cells ranged from 2.2 to 79.3%. We observed a linear correlation between results obtained by image analysis and visual scoring, despite threshold differences between both methods. With regard to intensity detection, a statistically significant correlation was also found between visual and automated scoring (Chi^2: 12.4, p-value: 0,002).

Conclusions: Our study shows a good correlation between visual and automated image analysis evaluation for both staining proportion and intensity of MMP-14 immunostaining of the epithelial component despite the high complexity of ovarian carcinomas. Studies are underway to compare visual and automated scoring with survival data.

1576 Diagnostic Challenges and Advantages of International Telepathology between Two Medical Institutions

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Background: 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 Califorrnia 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 the 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.

Conclusions: 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.

1577 Evaluation of Image Analysis for Interpretation of Her-2 Dual In Situ Hybridization (DISH)

JL Fine, DJ Dabbs, K McManus, R Bhargava. University of Pittsburgh, Pittsburgh, PA.

Background: Personalized breast cancer therapy includes evaluation for trastuzumab sensitivity, by testing for Her-2 gene amplification. Her-2 Dual in situ hybridization (DISH) is a test that combines visualization of Her-2 gene copies (in the context of chromosome 17 copies) with brightfield microscopy. Scoring Her-2 DISH is labor intensive, fatiguing, and slower than immunostain scoring. This is a great opportunity for automation and we report our evaluation of a newly available whole slide image (WSI) based image analysis (IA) system.

Design: Known Her-2 positive (22 TMA cores) and Her-2 negative (18 TMA cores) tissues were used. Her-2 DISH was performed per vendor's directions then slides were scanned as WSI at 40x magnification (iScan HT, Ventana, Tucson, AZ). Her-2 signal (black) and chromosome 17 signal (red) were scored using IA (Virtuoso, Ventana). If IA failed to auto-select nuclei, nuclei were manually circled; auto-selection was closely supervised and corrected as needed. IA errors were noted. Cases were eventually skipped if manual WSI preview showed low or no visible signal.

Results: Amplified cases were correctly identified in 100% of successful analyses (n=8), but only 47% of attempts were successful (n=17). Non-amplified cases were correctly identified in 12.5% of successful analyses (n=8), with 80% of attempts yielding a result (n=10). Many errors appeared to represent inability to "see" signal on IA, especially red signal. IA found nuclei automatically in 4 analyses and manual selection was performed in 23 analyses (n=27). In almost all cases, fewer than 10% of all selected nuclei generated a count result. 5 ampified and 8 non-amplified cases were not attempted after preview of WSI (inadequate signal).

Conclusions: True automation in surgical pathology remains elusive. IA had difficulty "seeing" low levels of signal, especially red signal. This caused over-calls, errors, and failure to auto-select nuclei. WSI did not compare favorably to 60x microscope image quality in terms of resolution and dynamic range. These issues were exacerbated by use of TMA cores, which contain fewer tumor cells and do not allow for individual pre-treatment of tissues. Additional validation using tissue sections may have better results. Numerous opportunities for workflow improvement were noted, especially when correcting or supervising nuclei selection. Despite these shortcomings, WSI-based interpretation of Her-2 DISH is highly desired; automation aside, the onscreen format is less fatiguing than microscope-based review and also potentially permits side-by-side comparison with the H&E slide.

1578 Approaches to Whole Slide Imaging for Surgical Pathology Consult Services

CS Friedman, L Rivera, Y-T Chen, V Brodsky. New York-Presbyterian Hospital/Weill Cornell Medical College, New York, NY.

Background: Whole-slide scanning is an effective method of overcoming logistical problems associated with consult services, such as slides being unavailable for review due to having been returned; but it also presents significant costs and storage requirements. As whole-slide scanning of consult cases was being implemented at NYP/Weill-Cornell, the question arose of whether all consult slides should be scanned, or only those selected by attending pathologists. The latter approach was adopted for five subspecialty consult services: General/Endocrine/Head & Neck/Thoracic (GEHNT), Gastrointestinal (GI), Gynecologic (GYN), Genitourinary (GU), and Neuropathology. The scanned cases were analyzed in order to evaluate usage patterns of a slide-scanning system under real-world conditions.

Design: Attending pathologists were instructed to submit representative slides for scanning at their discretion. During an 8-week period immediately following the initial launch of the whole slide scanning, representative slides from consult cases selected by pathologists for the GEHNT, GU, GI, GYN and Neuro services were scanned using the Aperio Scanscope AT (2012); Console version 101.0.4.413.

Results: Out of 210 consult cases, 92 were scanned, representing a scan rate of 43% overall. The cases contained an average of 9.40 slides each; 1 to 12 slides were scanned per case, averaging 1.98 slides per case. For the 5 consult services surveyed, the percentage of scanned cases ranged from 64% (56/87) to 0% (0/17). Of 14 pathologists who had received consult cases during the 8-week period, 9 submitted slides to be scanned. The scan rates ranged from 77% to 0% among pathologists. 49% of cases with neoplastic diagnoses were scanned versus 17% of non-neoplastic cases. Based on our previous data, scan time at "20X" averages 5.18 minutes per slide, and average file size was 498 megabytes (MB). Thus the consult cases required a weekly average of 1.96 hours of scan time and 113 gigabytes (GB) of storage. Scanning only selected slides from each case yielded savings of 59 hours of scan time and 340 GB of storage over an 8-week period.

Conclusions: The study revealed wide variations in scan rates between consult services as well as individual pathologists. The reason is likely multifactorial, including the different disease spectra encountered, the individual familiarity with the scanning service, etc. Non-neoplastic cases were less frequently scanned. Scanning representative slides from all consult cases, at the current rate of 1.98 slides per case, would result in a total of 35 hours of scan time and 207 GB of storage.

1579 Whole Slide Imaging Performance

CS Friedman, L Rivera, V Brodsky. New York-Presbyterian Hospital/Weill Cornell Medical College, New York, NY.

Background: Whole-slide scanning is becoming increasingly prevalent, but there is a lack of real-world data on the performance of whole-slide scanning systems. Our preliminary data had indicated that scan time (adjusting for the slides which need to be re-scanned and manual steps) at "20X" averages 5.18 minutes per slide and average file size was 498 megabytes; whereas at "40X" average time 27.58 minutes per slide, and average file size was 726 megabytes. As a scanning service was being implemented at NYP/Weill-Cornell, we analyzed scanner speed and workload for a larger sample of scanned slides, in order to enable institutions interested in scanning to define the time, personnel and storage requirements.

Design: Slides selected by attending pathologists were scanned using the Aperio Scanscope AT (2012); Console version 101.0.4.413. The slides comprised a mix of teaching slides, consult cases, and surgical pathology cases; the pathologists could choose to scan at "20x" (0.46 microns/pixel) or "40x" (0.22 microns/pixel).

Results: Over a 60-day period, 2433 slides were scanned; 2203 slides were scanned at "20x" and 230 were scanned at "40x". The average time to scan each slide at "20x" was 3.76 min. Factoring in the observed 2.5% rescan rate increased the time to 3.86 minutes. Including the pre and post-scan manual steps, which averaged 1.55 minutes per slide, the total average time per "20x" slide was 5.41 minutes. The average time to scan each slide at "40x" was 20.38 min. Factoring in the observed 5.5% rescan rate increased the time to 21.50 minutes. Including the manual steps, the total average time per "40x" slide was 23.05 minutes. Scanning at "20x" resulted in an average file size of 560 megabytes, and scanning at 40x resulted in an average file size of 605 megabytes. An average of 40.55 slides per day were scanned, with a maximum of 154 slides.

Conclusions: The scan times and storage requirements for "20x" slides increased compared with our previous data, whereas those for "40x" slides decreased. The decrease in average "40x" file size may be attributable to a large number of immunostained biopsy slides resulting in better compression ratios. We had previously calculated that a 7 hour work day of 1 full-time employee (FTE), followed by 16 hours of machine scan time can result in a predicted maximum of 260 slides scanned at "20x". Currently with 3.5 hours of daily FTE time 40.55 slides per day were scanned as we develop the scanning service. While scanning at "40x" significantly increased the scan time, having the attending pathologists choose the magnification resulted in only 10% of slides being scanned at "40x".

1580 Telecytology for On-Site Adequacy Evaluation Decreases the Nondiagnostic Rate in Endoscopic Ultrasound-Guided Fine-Needle Aspiration of Pancreatic Lesions

B Graber, A Roy, RD Szyjkowski, KK Khurana. SUNY Upstate Medical University, Syracuse, NY.

Background: In the recent years, the advances in digital methods in pathology have resulted in use of telecytology in the immediate assessment of fine needle aspiration specimens. We retrospectively compared the unsatisfactory rate for endoscopic ultrasound-guided (EUS) fine-needle aspiration (FNA) of pancreatic lesions in 2 situations: 1 with onsite telecytopathology evaluation and 1 without on-site evaluation.

Design: 216 consecutive adult patients underwent EUS FNA of pancreatic lesions. Direct smears were immediately wet fixed or air dried and any residual material was rinsed in saline for cell block or cytospin preparation. Patients were divided into 2 groups - one with telecytopathology on-site adequacy evaluation and other without any onsite adequacy evaluation. For the group with telecytopathology on-site evaluation, real time images of Diff Quik stained cytology smears were obtained with an Olympus Digital camera attached to an Olympus CX41 microscope and transmitted via ethernet to a pathologist who rendered preliminary diagnosis while communicating with the on-site operator over the Vocera system. The cytologic diagnoses were reviewed and the nondiagnostic rates for each group were calculated.

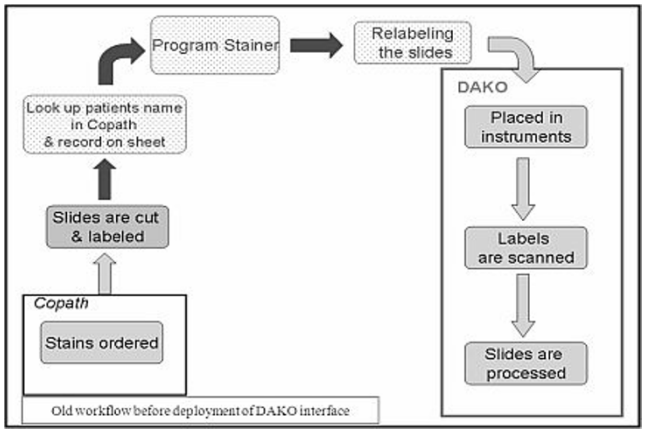
Results: Telecytopathology on-site evaluation was provided for 95 cases with a nondiagnostic rate of 4.1% (4 of 95 cases). On-site evaluation was not provided for 121 cases with a nondiagnostic rate of 19.8% (24 of 121 cases). Final cytologic diagnosis of unsatisfactory, negative/benign, atypical /suspicious and positive for malignancy were 4.1%, 40%, 17.8% and 37.9% for telecytopathology group and 19.8%, 62.8%, 9% and 8.2% for the group without on-site adequacy evaluation. The nondiagnostic rates and the percentage yield of malignant and suspicious lesions between the two groups were statistically significant ($P<0.005$).

Conclusions: Telecytopathology onsite evaluation of EUS FNA of pancreatic lesions reduces the non-diagnostic rate and yields higher percentage of malignant and suspicious lesions compared with the group without any onsite evaluation.

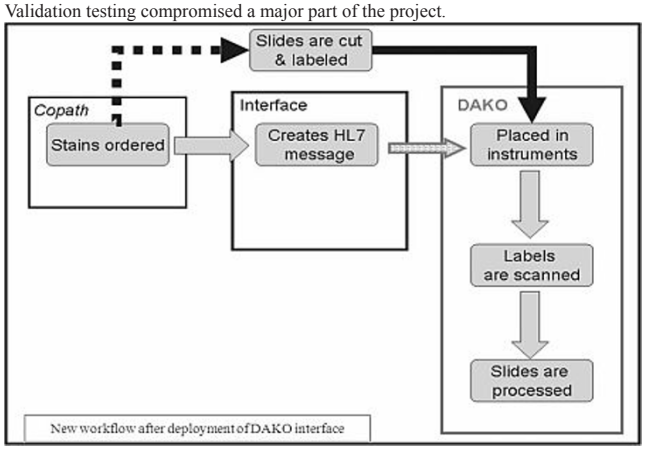
1581 Deployment of an Orders Interface between SunQuest CoPathPlus (SQCP) and Dako's Automated Immunoperoxidase Staining Platform

M Haghighi, M Czechowski, K Roszka, M Tuthill. Henry Ford Health System, Detroit, MI.

Background: Immunoperoxidase staining of tissues has become an important routine aspect of pathology practice resulting in the development of automation technology to perform these assays. As orders are created in the AP laboratory information system (AP-LIS), it is a logical next step to interface such orders to the automated instrument platform eliminating dual order entry and associated errors, improving efficiency, and thereby increasing capacity. Other key problems resulting from manual ordering are duplication of order entry and errors that direct the instrument to apply the incorrect primary antibody to a slide. By automating the ordering process errors will be eliminated and the order accuracy will increase.



Design: This project was performed in two phases.
Phase 1: A unidirectional HL 7 interface was implemented between Sunquest CoPathPlus 4.1(SQCP) and a Dako Immunostain Platform which allows immuno-histochemistry orders placed in CoPath to be received in the DakoLink software.
Phase 2: CopathPlus was upgraded to version 6.0 which provides the Unique ID technology and allows for CoPath to send the unique slide ID to Dako. This means that Dako can read CoPath native labels eliminating relabeling step of the process. Validation testing compromised a major part of the project.



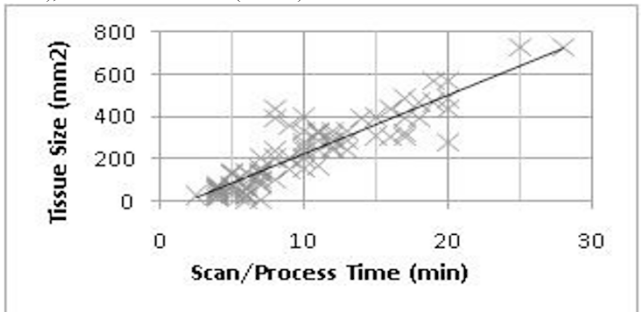
Results: The immediate impact of first phase was simplification of run setup. In second phase the relabeling step was eliminated which has led to further automation of the process and as a result increase in the saved time to 56 min per run for a total labor saving of 570 hours per year.
Conclusions: The elimination of dual order entry and relabeling the slides through automation markedly decreases assay run time, eliminates errors, and improves laboratory throughput.Also by reducing keystroke errors the order accuaracy will increase which would prevent the incorrect immunostain being applied to slide, with the potential for misdiagnosis.

1582 Automated Whole Slide Image Screening for Identification of Malignant Cytologic Criteria in Pancreactobiliary Brushings by Use of Spatially-Invariant Vector Quantization (SIVQ)

JA Hipp, JC Pang, UJ Balis. University of Michigan Ann Arbor, Ann Arbor, MI.
Background: Spatially-Invariant Vector Quantization (SIVQ) has exhibited promising results for facilitating detection of subtle architectural and nuclear features, making a compelling case for the exploration of its utility to screen (identify) adenocarcinoma from biliary brushings. Contemporary cytologic evaluation of pancreatobiliary brushings is specific (near 100%) but has a sensitivity of only 40-70%, with no absolute criteria for malignancy. Moreover, interobserver variability can be problematic. In this effort, we explore SIVQ's ability to provide a superior approach in analyzing atypia, including attaining improved sensitivity.
Design: Digital whole slide images were obtained from biliary brushings from 3 patients with a cytologic diagnosis of *suspicious for adenocarcinoma* with histologic confirmation of malignancy on resection. Use of a region-of-interest extraction tool followed by use of an image aggregation tool allowed for the generation of a montage of diverse cytologic features of adenocarcinoma, including nuclear enlargement, hyperchromasia, chromatin clearing, irregular nuclear membrane and macronucleoli. Given that nuclear enlargement with a malignant nuclear texture is one of the most specific morphologic criteria, two SIVQ detection vectors were chosen to identify nuclear enlargement with either hyperchromasia or chromatin clearing on high magnification.
Results: Vector I (specific for nuclear size and hyperchromasia) identified 10 malignant groups (sensitivity: 62.5%, specificity: 100%) while Vector II (specific for nuclear size and chromatin clearing) identified 9 malignant groups (sensitivity: 56.3%, specificity: 100%). Both vectors were chosen to be orthogonal in their detection features; when combined, 15 of 16 malignant groups were selected (sensitivity: 93.8%, specificity: 100%).
Conclusions: Combined, multi-vector-based SIVQ detection is able to efficiently identify the nuclear features of adenocarcinoma, with a high sensitivity and specificity, while avoiding false-positive detection in areas of benign groups. This study provides pilot data demonstrating SIVQ as a suitable means to address the challenge of automated digital screening for cells suspicious for adenocarcinoma. Future studies are being considered to address the need for inclusion of multiple cytologic criteria for malignancy as applied to the cytologic diagnostic category of atypical, as this population represents the greatest uncertainty for accurately and consistently diagnosing malignancy.

1583 Low-Cost Whole Slide Imaging for Routine Frozen Section Diagnostics

AJ Horn, JJ Baker, MF McGaughey, KW Foster, AJ Lazenby. University of Nebraska Medical Center, Omaha, NE.
Background: As the technology of whole slide imaging (WSI) has matured, there has been a reduction in size/cost of the instruments allowing for adoption at varied sites. Previous studies have evaluated WSI for the diagnostic accuracy of frozen sections (FS) for limited specimen types. Little has been published on the pre-analytic issues of WSI for routine FS use on all specimen types. We investigated a low-cost desktop whole slide imager for routine FS diagnosis of all specimen types, evaluating pre-analytic/analytic time, diagnostic accuracy, and technical issues.
Design: 35 FS specimens from 1 month were randomly selected. The archived H&E cryoblock slides (95 slides) were digitized at 20x using a low-cost desktop whole slide scanner. Tissue area (mm^2) was calculated using the viewing software. Scan and post-scan image processing time was recorded. De-identified surgical requisitions with clinical information were provided to two senior surgical pathologists who reviewed the scanned slides on a single designated computer/monitor. The diagnosis, interpretation time, and comments were recorded.
Results: The average combined scan/process time was 9.8 min/slide and 26.6 min/specimen, ranging from 2.5-28 min/slide. The average tissue size was 216.3 mm^2 with an average scan/image process time of 21.9 mm^2/min. The scan time is directly proportional to the tissue size (Fig 1, r^2=.78). The average interpretation time was 1.2 min/slide and 3.4 min/specimen. The concordance with the glass slide diagnosis was 97% for pathologist 1 (missed micromet in lymph node, 1 deferred) and 91% for pathologist 2 (missed micromets in lymph node and soft tissue). Technical issues included slides being dark/thick (6 slides), scanned tissue cut off near edge of slide (21 slides), and tissue out of focus (7 slides).



Conclusions: 1) WSI for FS interpretation yields good-excellent diagnostic accuracy. 2) Analytic time is similar to light microscopy. 3) Using a currently available desktop scanner, scan time is very long for large specimens, which may limit usefulness in routine practice. 4) Slides of large pieces of tissue prepared via cryostat sometimes yield thick, folded sections with tissue placed at the edge of a slide, and thus there are more technical issues with WSI (tissue cut off, out of focus) compared to paraffin.

1584 Efficient Donor Tissue Assessment through Integrated WSI and LIS for a Regional Organ Donor Bank: An Untapped Application of Virtual Microscopy

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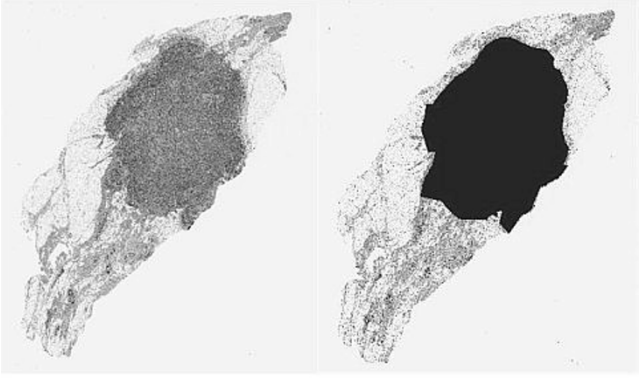
Background: Organ harvesting for transplantation is complex and involves diverse locations, donor selection, organ procurement and preservation, tissue matching and donor tissue assessment for transplant worthiness. While most other steps are performed by trained donor bank staff, urgent tissue assessment involves frozen sectioning and evaluation by a pathologist. This step could be rate limiting due to delay in tissue transport and processing, unavailability of expertise and lack of time for consultation. Virtual microscopy, through integrated whole slide imaging (WSI) and laboratory information system (LIS), addresses these issues and extends readily available electronic data to all the organ receiving centers in the network.
Design: From June 2011 through August 2012, 142 cases of bilateral renal donor tissue and were subjected to around-the-clock frozen sections and WSI and uploaded to an integrated LIS in a regional organ donor bank's centralized laboratory serving parts of two states. All cases were immediately and remotely reviewed and reported via virtual microscopy by 1 of 6 University medical center pathologists on-call, well-versed with virtual microscopy. The LIS had integrated WSI, donor clinical data, fillable kidney biopsy reporting templates and in selected cases, gross images of the organs. After the organ was either utilized or discarded, the glass slides were subsequently reviewed (within one week) by an expert renal pathologist for validation of WSI assessment.

Results: There was total concordance between WSI and subsequent expert glass slide review with no impact on organ utilization (100% agreement). The reasons for such concordance included ready availability of clinical data, systematic and detailed enforced entries into templates creating consistent and reproducible reports, high quality of scanning and comfort-level between pathologists for virtual microscopy. No case was rejected due to inability to read WSI. All state-wide centers utilizing the donor organ had the ability to remotely view the entire donor data including WSI and seek instant additional consultation if needed.
Conclusions: Integrated WSI, LIS and virtual microscopy is highly effective and efficient in an organ donor network setting. This untapped application of virtual microscopy addresses both the geographic and time constraints inherent in organ donation process. Furthermore, at the points of organ utilization, the entire clinical, laboratory and tissue electronic data is available for instant reappraisal.

1585 Computer Vision Methods in Surgical Pathology: Diagnosing Carcinoma of the Breast

A Kaplan, X Li, R Sivalingam, G Somasundaram, A Banerjee, V Morellas, N Papanikolopoulos, A Truskinovsky. University of Minnesota, Minneapolis, MN.
Background: Based on our successful application of computer vision methods to detect endometrial and prostatic adenocarcinomas, we extend our analysis to ductal carcinoma of the breast, using region covariance descriptors.
Design: We used 5 images each of H&E-stained sections of 2 diagnostic classes, carcinoma and benign breast, scanned at x50 magnification on a digital slide scanner. The color images were transformed to grayscale using a custom transformation and then manually annotated to train the classification algorithm. For this study, we have developed a new Graphical User Interface (GUI) to enable a pathologist to annotate tissue images for computer analysis. The annotated regions are then broken down into overlapping blocks of 150 x 150 pixels. Each block is represented by the covariance matrix over the image features in that block. We used a set of spatial (x, y, ρ, θ) and intensity [I, Ix, Iy, √(Ix2+Iy2)] features, giving rise to 8x8 region covariance descriptors (RCDs). Test image patches are classified via k-NN classification using a geodesic distance measure over RCDs. We ran a 10-fold cross-validation on the entire dataset to test performance on unseen images, where we randomly select one tenth as test images from each class, using the rest for training.
Results: Using RCDs with more than 6000 labeled training patches, the overall classification accuracy of the image blocks into cancerous vs. benign regions was 98%. Figure 1 shows a ductal carcinoma on the left, and the same tumor successfully identified and highlighted by the algorithm on the right.



Conclusions: We have demonstrated that computer vision methods can be applied to diagnose ductal carcinoma of the breast with high accuracy. The approach based on RCDs has been previously shown by us to be accurate for endometrial and prostatic adenocarcinomas. The continued success on carcinoma of the breast indicates that RCDs are indeed robust as features for cancer diagnosis. This study also highlights the successful use of our new image annotation GUI. It is simple and intuitive and does not require advanced computer knowledge to operate. Using this GUI, the computer vision algorithms can be trained for new tasks and applied to more and more fields of diagnostic histopathology.

Abstract #1586 moved to Breast

1587 Comparison of Manual Count and Image Analysis Methods in Reporting Ki-67 Index as Prognostic/Predictive Marker for Breast Cancer

LH Lee, G Bigras, RT Ogilvie, H Yang. University of Calgary, Calgary, AB, Canada; University of Alberta, Edmonton, AB, Canada.
Background: Ki-67 index is a known important prognostic/predictive factor in breast cancer and is increasingly requested by oncologists. However, the accuracy of manual Ki-67 evaluation is questionable with significant inter-observer variability and intra-tumour heterogeneity. Additionally, it is time consuming in an era of cost cutting and increased pathology workload. Thus, there is interest in automated digital assessment of Ki-67 to increase precision, accuracy, and efficiency. The objective of this study is to compare automated image analysis and manual counting of Ki-67 in invasive breast carcinoma.
Design: Cases of invasive ductal carcinoma with Ki-67 index requests at our institution were identified. A breast pathologist reviewed the cases and selected the block with highest mitotic activity. A slide was made and stained for Ki-67 (Dako MIB-1 stain at 1/200 dilution, DAB chromogene) with haematoxylin counter-stain. The Ki-67 index was reported as the average independent count of two breast pathologists. The slides were then digitized using a Nikon Eclipse E600 microscope (20X objective, 0.40 aperture) with a QImaging MicroPublisher 5.0 camera (24-bit color, 2560 x 1920 pixel sensor). *A priori* background correction was applied. Three images targeting the most proliferative areas were analyzed with the ImmunoRatio application plugin in the ImageJ environment. The software identified carcinoma nuclei based on nuclear circularity and calculated the DAB-to-nuclear area ratio for the Ki-67 index. Manual and automated analysis results were compared with the paired samples t-test.
Results: 21 cases of were identified. There was a significant difference in the Ki-67 index between manual count (M=29.4, SD=13.2) and automated count (M= 19.0, SD=9.8; $t(20)=6.22$, $p<0.001$). The automated count was lower than the manual score (mean difference = 10.5, 95% CI=6.1 to 14.0) in all but one case, which was equal.
Conclusions: Automated assessment provides a consistently lower Ki-67 index than manual assessment. We postulate that this is due to the software algorithm used to identify neoplastic epithelial cells. Non-tumour elements, which usually do not stain for Ki67, may be mistaken as tumor, including benign epithelial cells, lymphocytes, and visual artifacts. With improved techniques or algorithms to isolate neoplastic epithelial cells, automated Ki-67 accuracy can be improved, potentially making it a viable alternative to manual Ki-67 assessment.

1588 Utilizing Image Analysis in the Assessment of Decalcification Effects on Prognostic Breast Markers

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Background: Treatment options for breast cancer bone metastases are often determined by the immunohistochemical expression of predictive biomarkers- Estrogen (ER), Progesterone (PR), Ki-67, HER-2/neu and p53. Bone biopsies require decalcification prior to histologic evaluation, however the effects of decalcification on expression of biomarkers are unknown. Using an experimental model and time course, this study is designed to quantitatively assess whether or not decalcification affects the results of the biomarker measurements.
Design: Residual tumor samples were prospectively collected from 9 breast cancer specimens: 1 grade 2 and 8 grade 3 invasive ductal carcinomas. Samples were formalin fixed according to CAP guidelines, and placed in an acidic decalcification solution (Decal State, Decal Chemical Corp., Tallman, NY) at ambient temperature for 0, 1, 6, and 24 hours(hrs). After routine processing, sections were stained by immunohistochemistry utilizing the Ventana System (Oro Valley, AZ). Stained slides were digitized at x20 magnification on a Leica SCN 400 (Buffalo Grove, IL) scanner. Tumor areas were randomly selected and image analysis was executed using the Tissue IA 2.0 (Leica) software. After delineating cell membranes and nuclei, signals were quantified in subcellular compartments. Numerical scores were converted to categorical scores of weak, moderate and strong to recreate the conventional reporting of biomarker expression by pathologists.
Results: Upon image analysis, the signal intensities and percentages of positive cells gradually decreased with the length of decalcification and a significant decrease was observed after 6 hrs for all markers. Progressive loss was further confirmed microscopically and noticeable at decalcification times ≥ 1hour. It correlated with a significant reduction in the percentage of positive cells. No increase in immunoreactivity was observed for any of the markers after decalcification.
Conclusions: Decalcification reduces the immunoreactivity of predictive breast cancer markers. The time of decalcification may endanger optimal treatment decisions by causing artificially low protein expression signals. This project demonstrates that image analysis can provide an objective and precise assessment of the adverse effects of decalcification on biomarker measurements and can lead to the development of a strategy for correcting the loss of immunoreactivity in decalcified tissues.

1589 Conversion of Traditional Immunohistochemistry into Virtual Multicolor Stains Using Feature Extraction and Pixel-Conversion Algorithms

A-SK Meyer, P Moller, JK Lennerz. Ulm University, Ulm, Germany.
Background: Colocalization analysis is too time-consuming and cost-intensive for routine diagnostics. Therefore, immunophenotyping is -for the vast majority of clinical assessments- performed via mental combination of single stains. Here we present a set of pixel-conversion algorithms that generate a synthetic image for marker covisualization and/or subsequent quantification.
Design: Single immunohistochemical (IHC) stains were digitized and subsequently either re-stained and re-digitized or merged with separately digitized subsequent sections. The applied algorithms are composed of the following components: a user-defined feature extraction, a color conversion and an overlay component. To allow downstream analysis a customized link between ImageJ, Photoshop CS3 (Adobe Systems), and ImageScope (Aperio) or OlyVIA (Olympus) was generated using AutoIT (version 3.2.12.).
Results: The algorithms emulate multicolor staining without additional use of tissue or equipment and preserve the immunohistochemical stain for traditional assessment. The extracted and converted elements enable covisualization on digital merges and/or subsequent quantification of co-localized elements using established methods. The algorithms solve the common problem of species identity and/or cross-reactivity of primary antibodies because they allow co-visualization of markers without compromising staining specificity. When compared to investments in immunofluorescence microscopy equipment and validation of traditional multi-color stains, the effort to learn and perform the conversion algorithms is negligible.
Conclusions: The presented imaging tools emulate the principal advantages of multi-color fluorescence microscopy as well as multi-color immunohistochemistry without

compromising traditional assessment. Thus, the algorithms expand the versatility of one of the most important molecular tools in diagnostic pathology (i.e. IHC) and allow visual integration that exemplifies the potential of digital pathology.

1590 How Digital Image Analysis Applied to Immunohistochemistry Helps To Assess Biomarker Coexpression

X Moles Lopez, P Barbot, N D'Haene, C Maris, S Rorive, I Salmon, C Decaestecker. Université Libre de Bruxelles (ULB), Brussels, Belgium; Center for Microscopy and Molecular Imaging, ULB, Gosselies, Belgium; Erasme Hospital ULB, Brussels, Belgium.

Background: Immunohistochemistry (IHC) has allowed identification of various tissue biomarkers with diagnostic, prognostic or theranostic values. However, pathologies such as cancers combine complex interactions of different pathways, which make the study of colocalization of multiple markers of great importance (e.g. to identify cell types expressing different biomarkers). However, multiple IHC has technical limitations so that this technique is not suitable in clinical practice. Consequently, new methods to evaluate colocalization of multiple biomarkers are needed.

Design: We developed an original approach to combine information provided by multiple IHC stains on serial tissue sections. This integrated approach combines standardized IHC, whole slide scanning and digital image analysis. Expression patterns are colocalized using a two-step, multiscale, image registration procedure that was chosen for its capability to register extremely large images.

Results: This multiscale approach starts by registering pairs of images downsampled to a very low resolution (approximately equivalent to a 0.13X objective) and then refines the registration transform for gradually increasing resolutions. During the first step, resolutions are increased up to a resolution corresponding to a 2X objective (chosen as a compromise between the execution time and the registration precision). However, this step does not allow a valid colocalization of IHC stains at the histological structure scale. It is thus completed by a second step where the corresponding regions of interest (extracted from the two images) are finely registered with an increased resolution (corresponding to a 20X objective). Our approach is qualitatively and quantitatively validated using checkerboard visualization and pathologist supervision.

Conclusions: This method enables to characterize particular cell populations or to test multiple biomarkers and identifies with greater accuracy cellular heterogeneity in tissue samples. Our goal is to apply this methodology to characterize colocalization of proliferation marker (Ki67) and expression of growth factor receptors (IGF-1R, EGFR) in glioblastomas.

1591 Live Streaming Using Consumer Level Technology

M Nassiri, MB Czader. Indiana University School of Medicine, Indianapolis, IN.

Background: In daily clinical hematopathology practice there is a need for sharing the microscopic findings with clinical hematologists. However in large medical centers with centralized laboratories, physical attendance is cumbersome for clinicians. Furthermore, despite advances in whole slide imaging, this technology is expensive, not instantaneous and cannot handle wet mounted slides easily.

Design: High definition AXIS P1347 network cameras (Progressive scan RGB CMOS 1/2.5", Lund, Sweden) were mounted on Olympus BX-41 microscope's c-mount. The camera has a built-in web server and allows an output up to 2560 x 1920 pixels and frame rate up to 30 frames per second (fps). Outputs of streams from different cameras at different Indiana University Health (IUH) locations were linked through a simple html portal page and hosted on a virtual server in a private IP network. Users could access the portal across the IUH network in Indiana or through VPN. Privileges were maintained through LDAP (Lightweight Directory Access Protocol).

Results: Portals and cameras ran continuously for the last 18 months, streaming at 1920x1080 pixels at 30 fps, with minimal maintenance and no failure. Lower resolutions, down to 640x480 pixels were acceptable to clinicians who wanted live review of the slides and pathologists. There was no difference in user experience based on different video compression protocols (H.264 or motion JPEG). Cost of implementation was limited to the cost of the cameras. Images captured at high resolution were used in interdisciplinary conferences and were acceptable for publication purpose in peer reviewed journals.

Conclusions: Live streaming using consumer level technology is a low cost and viable method for real-time demonstrations of microscopic findings to clinicians in large multicenter institutes.

1592 Digitized Whole Slides for Breast Pathology Interpretation: Pathologists Use and Perceptions

T Onega, KH Allison, N Ostler, J Elmore, DL Weaver. University of Washington, Seattle, WA; Dartmouth School of Medicine, Hanover, NH; University of Vermont, Burlingame, VT.

Background: Digital whole slide imaging (WSI) is an emerging technology for pathology interpretation. Little is known about patterns of use among pathologists or their perceptions of WSI for breast pathology interpretation. This study characterizes use of WSI among a national sample of pathologists and examines their perceptions of this technology.

Design: We surveyed a national sample of eligible pathologists from New Hampshire, Vermont, Washington, Oregon, Arizona, Alaska, Maine, and Minnesota in this cross-sectional, descriptive study. Pathologists' self-reported professional characteristics and WSI practice patterns and then responded to perception statements about WSI using a 6-point Likert Scale. Descriptive analyses were used to summarize and characterize pathologists' use and perceptions of WSI.

Results: Ages of the 175 pathologists surveyed to date were: <40 (11%); 40-49 (32%); 50-59 (38%); and ≥60 (19%) years. 26% of pathologists surveyed were affiliated with an academic medical center and 74% were not. Utilization of WSI was reported by 46% of pathologists. Types of use reported included: CME/board exams/teaching (28%), tumor board/clinical conference (18%), archival purposes (5%), consultative diagnosis (3%), research (2%), primary pathology diagnosis (1%), and other uses (11%). 9% used WSI for IHC interpretation and <1% for H&E diagnosis. Answers to questions on perceptions of WSI suggest that most agree that accurate diagnoses can be made with this technology (79% agree), and that WSI is useful for obtaining a second opinion (86% agree). (Figure 1) At the same time, 80% of pathologists agree that digital slides are too slow for routine initial interpretation. Potential barriers to use of WSI such as increased exposure to medical malpractice suits and difficulty learning how to use WSI did not appear to be of great concern for most pathologists. When asked if the benefits of WSI outweigh the concerns, 60% agree, and 40% disagree. Half of pathologists agree they would like to adopt WSI (52%) while the other half disagree (48%).

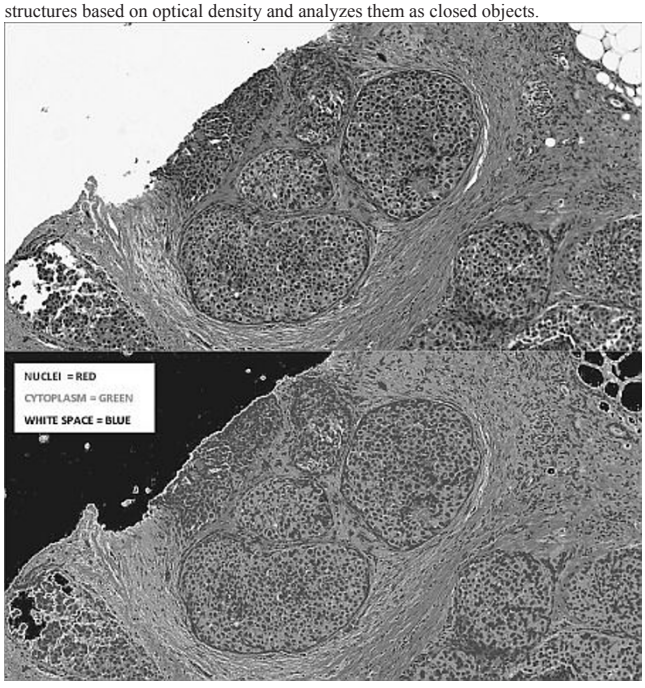
Conclusions: While the majority of pathologists do not currently use WSI, perceptions towards this technology are somewhat positive. Pathologists' current use of WSI is largely for CME, licensure/board exams, and teaching. Practice patterns for this emerging technology should be tracked as dissemination advances.

1593 Morphometric Analysis in the Diagnosis of Ductal (DCIS) and Lobular Carcinoma In Situ (LCIS)

C Parra-Herran, T Hope, E Slodkowska, F-I Lu, T Rashed, M Katic, G Han, G Clarke, M Yaffe, S Nofech-Mozes. Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada; Centre for Imaging Technology Commercialization, Toronto, ON, Canada.

Background: Distinction between DCIS and LCIS, while critical for margin assessment and further management (re-excision, radiation), is sometimes challenging. Immunohistochemistry for E-Cadherin and Catenin P120 has proven utility. However up to 15% of LCIS cases show E-Cadherin expression. We aim to identify quantifiable histologic features by digital image analysis that can differentiate between DCIS and LCIS as an adjunct diagnostic tool.

Design: Scanned images of 13 cases of DCIS and 14 cases of LCIS were analyzed. Three regions of solid intraductal proliferation were selected from each case and analyzed using Mercator Version 1.0 (ExploraNova, La Rochelle, France). The system detects structures based on optical density and analyzes them as closed objects.



We analyzed multiple morphometric variables.

Morphometric variables in DCIS and LCIS

VARIABLE	FORMULA
Nuclear density (ND)	NC/TSA
% Nuclear surface area	SNA/TSA*100
Mean nuclear area (MNA)	SNA/NC
Mean nuclear form factor (MNFF)	SNF/NC
% direct cytoplasm area (measured by the system)	SCA/TSA*100
% indirect cytoplasm area (calculated)	(TSA-SNA-SWS)/TSA*100)

NC = Nuclear count, TSA = Total Region Surface Area, SNA = Sum of all nuclear areas, SNF=Sum of nuclear form factors, SCA = Sum of all cytoplasmic areas, SWS = Sum of all white space areas. All areas in μm2.

Non-parametric Wilcoxon Mann Whitney test was used to evaluate the difference between the two groups.

Results: Four variables analyzed were significantly different between LCIS and DCIS. LCIS showed higher nuclear density (0.01301 vs 0.01003, p=0.0066), higher nuclei surface area (34.67049 vs 24.07501, p=0.008) and lower direct and indirect cytoplasm surface area (1.42364 vs 13.27139, p<0.0001; 64.9085 vs 75.33277, p=0.0084) compared to DCIS. Nuclear size and shape (given by the MNA and MMFF respectively) showed no significant difference.

Conclusions: Morphometric features related to nuclear density and amount of cytoplasm are different between DCIS and LCIS. This correlates with the standard qualitative criteria conventionally used. Our study underscores the potential of measurable variables using image analysis as adjunct diagnostic tools in the era of digital pathology.

1594 Design and Implementation of a Model Digital Pathology Network for Teleconsultation and Workload Distribution within the Air Force Medical Service

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Background: Air Force Medical Service (AFMS) is exploring ways to introduce digital pathology (DP), utilizing whole slide imaging (WSI), into its pathology labs. As part of a research project, UPMC is supporting the design and implementation of a model DP network within AFMS.

Design: The model DP network would support remote sharing of digital slides between 4 AFMS regional and 2 smaller pathology labs distributed across the US. To design the DP network, unique needs and requirements of AFMS pathologists and pathology organization were identified using the contextual inquiry method by Holtzblatt et al. Findings were utilized to recommend most appropriate clinical applications. The network would be comprised of US commercially available and serviced WSI scanners. Following installation of scanners and successful connection to the AFMS intranet, implementation of DP across the recommended clinical application/s will be established. DP adoption and utilization will be tracked and evaluated.

Results: Contextual inquiry findings were reported previously (Ho J et al. USCAP 2012). The recommended clinical applications were consultations, quality assurance, and global workload distribution. A comprehensive review and comparison of WSI scanners/systems was performed and included scanner/system technical features, image quality, and usability. A single preferred WSI system was selected and installed at all participating sites. The clinical application currently being implemented is a virtual conference of AFMS subspecialists located at various pathology centers (i.e., dermatopathology, oral pathology). Other applications to be established are informal/formal subspecialty consultations and potentially centralization of immunohistochemical stains.

Conclusions: Introduction of DP into a large healthcare organization such as AFMS requires careful design and implementation to ensure successful adoption and utilization. Implementation of the model network and lessons learned will provide a valuable framework to help guide AFMS pathologists prepare for an innovative and streamlined DP practice.

1595 Evaluation of Whole Slide Imaging Systems Prior to Establishing a Model Digital Pathology Network for the Air Force Medical Service: Methods and Selection Criteria

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Background: Prior to acquisition of whole slide imaging (WSI) scanners to establish a model digital pathology (DP) network within Air Force Medical Service (AFMS), a comprehensive overview of commercially available WSI scanners was conducted.

Design: To be considered as potential candidates for acquisition, WSI systems had to support sharing of non-cytology anatomic pathology digital slides between multiple geographically remote AFMS pathology centers. Systems had to support select clinical applications/workflows and become security certified by the Air Force and Department of Defense. Evaluation criteria and features were identified and utilized to support scanner selection process.

Results: To match AFMS needs WSI systems had to meet the following key criteria: 1. high-speed scanners providing high-resolution high-quality digital slides; 2. manufacturer/ distributor presence in the US (operations, sales, customer service support); 3. usability; 4. scalable throughput to support network expansion. Scanners that met these criteria underwent comprehensive evaluations and manufacturers were invited to demonstrate their capabilities at a major pathology conference. Objective criteria for the comprehensive evaluations included a select set of technical features, including: scanner slide capacity, scanning capabilities (method, resolution, speed, Z-stack, automation, florescence), optics (camera, objective lens), integration with LIS, and others. During the conference, subjective criteria such as image quality and usability were evaluated. Identical sets containing six preselected glass slides were scanned by each system (prior to the meeting). All images were provided for review via the web and via a locally installed image viewer on a dual-screen workstation. Following demonstrations and image viewings, usability of each scanner and its supporting software (ie, image viewer and data management) was evaluated by AFMS pathologists. Usability evaluation was based on System Usability Scale developed by Brooke et al.

Conclusions: To support acquisition and selection of WSI scanners for an organization/lab, both objective and subjective criteria should be selected for the evaluation process. Pathologist participation is essential to the decision-making process.

1596 Virtual Slides in Surgical Pathology Practice – Keep Them or Toss Them?

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Background: As whole slide imaging (WSI) gains acceptance in the surgical pathology clinical workflow, the issue of virtual slide storage will become the primary hurdle to adoption. Virtual slides range up to 9GB in size, depending on the scanner and compression used on acquisition. Given that surgical pathology labs generate tens to hundreds of thousands of slides annually, storage requirements could approach up to a petabyte of data storage per year. Unlike radiology, which operates clinically on a "write once, read often" data storage model, pathology has been postulated to operate on a "write once, read once" model, an assertion that has not been formally evaluated. This study assesses the clinical usage of surgical pathology archives during the signout process.

Design: Seven pathology trainees (five residents and two fellows) recorded the number of prior cases and their respective slides pulled from the pathology archives during the clinical signout process with attending pathologists. Information for each case pulled included: 1) age of prior material; 2) number of slides pulled; 3) number of slides viewed; and 4) number of times each slide was viewed (trainee and attending). Data analysis of the surveys was performed in Microsoft Excel.

Results: Over thirteen work days, the participants signed out 791 cases, from which in 17 cases prior material was requested (2.2% of all cases). Prior archival material was pulled in 13 of 17 cases (1.6% of all cases). Archival material was viewed in all 13 instances it was pulled. Of a total of 172 slides pulled, only 24 slides were used for review (14.0% of slides pulled, 1.8 slides per case pulled). Given that each slide was reviewed by multiple individuals (slide-views), there were 50 slide-views of prior case materials (3.9 slide-views per case, 0.55 slide-views per work day). Prior cases had a mean age of 672 days (median age 62 days; range 13 to 5874 days). Trainees pulled 92.3% (12/13) of prior cases before sign-out with attendings.

Conclusions: From the data above, surgical pathology does indeed seem to fall in a "write-once, read-once" model of data persistence – pathologists rarely need to go back to prior case material during clinical signout. With the considerable data storage requirements for WSI, the argument for permanent persistence of virtual pathology slides (similar to radiology) seems unwarranted at this time. However, given the median age of prior case material, a temporary persistence model may be reasonable solution.

1597 Open-Source Three-Dimensional Digital Pathology: Application to Serially Sectioned Renal Needle Core Biopsies

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Background: Evaluation of microscopic three-dimensional (3D) structure of tissue has great potential in anatomic pathology. Currently evaluation of serial sections to generate a "3D" data set, e.g. medical renal biopsies, are performed manually on 2D tissue sections from which the 3D structure is inferred. With advances in whole-slide imaging (WSI), pathologists now have the opportunity for digital 3D analysis. The renal parenchyma is complex and geometric, and stratified and compartmentalized at all levels. A digital 3D construct would provide insights difficult to appreciate on 2D views.

Design: We have developed software tools for aligning WSI of serial tissue sections, and for viewing the resulting large-scale 3D image datasets. These tools are integrated into a web-based digital pathology system that will be released open source. The alignment algorithm differs from existing solutions in that it is designed to handle the artifacts typical of tissue sections including warping, folding, tearing and loss of tissue. To minimize needed server resources, field transformations are stored with the original (unmodified) image data and applied using a WebGL viewer. The system uses a load-on-demand strategy and progressive rendering to ensure the WebGL viewer remains responsive over standard networks. With this approach users are able to interactively navigate large volumes (i.e., quickly step through sections, pan, zoom and rotate).

Results: We have generated 3D image volumes from serial sections of renal core biopsies. Here we present an example from one full biopsy sectioned at ~2 microns to generate 66 tissue profiles on 23 slides (2 to 4 tissue profiles / slide). Sections were first roughly aligned and then finely aligned (Figure 1). The aligned whole slide images were then annotated and individual components measured and evaluated.

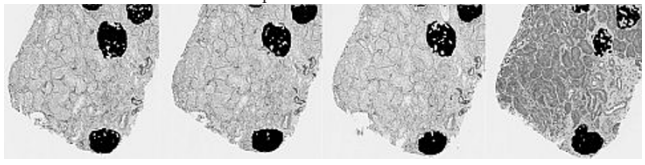

Figure 1: For fine alignment glomeruli are detected using texture classification

Conclusions: Using our software and alignment algorithm we are able to generate 3D image volumes from routinely processed renal biopsies. This shows the feasibility of 3D image modeling from 2D images from any part of the renal parenchyma. Examples will be shown.

1598 Challenges in Establishing the WSI Based Digital Pathology Facility and Telepathology Network between Pakistna and USA

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Background: In Pakistan, the number of physicians is not conversant with the number of patients. When it comes to pathologists, the situation is worse and there are serious deficiencies of pathologists even at tertiary care hospitals. This project has been started in 2007 to implement a modern digital pathology facility at a Holy Family Hospital (HFH), Pawalpindi, Pakistan working with a group of Massachusetts General Hospital

(MGH), Boston, US. However, because of situations around the world, it was not easy to perform the project smoothly. After overcoming many challenges, we have established the telepathology network between MGH and HFH in 2011 and then the 1st Whole Slide Imaging (WSI) scanner in Pakistna has been installed. Current project goal is that MGH supports HFH to be able to support other facilities in Pakistan using the digital pathology. In the paper, we focused on training, establishment of technical and clinical network between two institutions and the implementation of a WSI scanner at HFH.
Design: A pathologist at HFH has spent for two weeks at MGH for the digital pathology training and attending the signing out sessions and conferences. Prior to the pathologist visiting, MGH prepared all necessary equipment and all required software have been installed and tested. After the pathologist returned with equipment to Pakistan, the weekly static image based teleconference has been scheduled. Screen sharing software was selected for teleconference. Also MGH could access the scanned WSI using remote access software prior to the teleconference.
Results: The training sessions for pathologist from HFH were very successful and useful for the project. Starting December 2011, a weekly conference for 1 hour between MGH and HFH has been performed. Pathologist at HFH presents 7-10 variety cases using PowerPoint. All sessions have been recorded as a video and PowerPoint file. The details of WSI based teleconference will be reported. During the conference, we do not use any of patient identifier.
Conclusions: It was most useful for us to have the pathologist at MGH for two weeks. To learn the differences of culture, pathology practice, IT environment, and having communication directly helped us to continue teleconference smoothly and also made us easier to support from US. Working with pathologist and Telemedicine center at HFH, this project is going to be extended to other institution in Pakistan.

1599 Lymph Node Metastasis Status in Primary Breast Carcinoma Can Be Predicted Via Image Analysis of Tumor Histology

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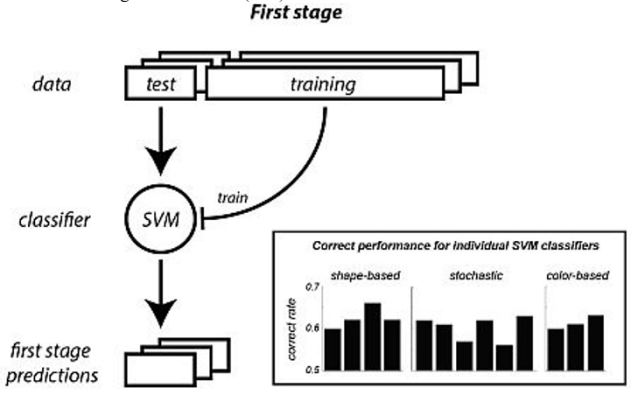
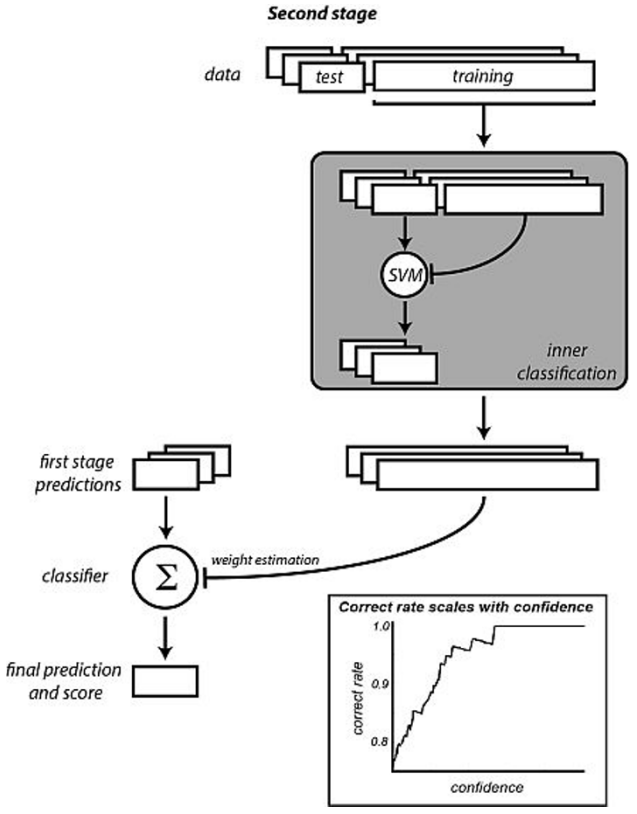
Background: Axillary lymph node metastasis status remains one of the most critical prognostic variables for breast cancer management and patient survival. Methods to improve reliability must be developed to avoid unnecessary surgeries and complications. The objective of our study is to demonstrate that lymph node metastasis status may be predicted via computerized image analysis of primary breast tumor histology.
Design: High-resolution (0.5µm/pixel) whole-slide images were produced from primary breast carcinoma specimens stained with a complete prognostic panel. Cell structures were extracted from these images using a custom segmentation paradigm. The properties of these structures were analyzed using stochastic geometric and chromatic transformations, forming a set of feature distributions that characterized the attributes of the cells in each sample. We constructed predictions individually for each feature distribution using a support vector machine classifier (SVM), and formed a final prediction from the individual predictions in an adaptive manner. Each prediction was accompanied by a confidence score, allowing us to relate the accuracy of the prediction to the certainty of the classification.
Results: We found that this procedure is highly predictive of axillary lymph node metastasis. The system achieved >95% correct rate for the highest scoring cases, which comprised over half the total number of cases we evaluated. Analysis of these cases revealed that N0 predictions correlated with T1 tumors (<2cm) of histologic Nottingham grade (HNG) 1 with low Ki-67 scores (<10). N1 predictions revealed T2/3 tumors of HNG 2/3 and high Ki-67 scores (>20).





Conclusions: We have demonstrated a fully automated procedure to predict metastasis status from histopathological images.

Kidney

1600 Methylmalonic Acidemia: A Megamitochondrial Disorder Affecting the Kidney

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Background: Methylmalonic acidemia (MMA) is an inborn error in the catabolism of branched-chain amino acids and is caused by mutations in the methylmalonyl-CoA mutase (MUT) gene. The MUT enzyme is located in the mitochondrial inner space and has a requirement for 5-deoxyadenosylcobalamin. Although MUT is expressed in many tissues, including the liver, brain and kidney, the patients experience organ specific disease such as basal ganglia dysfunction, pancreatitis and chronic kidney disease. As survival in MMA has increased, renal disease has become very important. Animal models have suggested that mitochondrial dysfunction with megamitochondria formation in selected cell types might contribute to specific disease manifestations but the lack of supporting studies has hampered efforts to discern the pathological features of MMA.
Design: Here we describe light and ultrastructural studies on the native kidney and liver from a 19 year-old patient with vitamin B12 non-responsive MMA, who underwent a combined transplant. She presented as a newborn with coma, metabolic ketoacidosis and massive hyperammonemia; severe MUT deficiency was identified. Stable in the first decade of life, she later developed progressive renal dysfunction.
Results: Kidney sections showed proximal tubular megamitochondria (proximal tubule vacuoles by light microscopy). In electron microscopy studies, the most dramatic changes were restricted to proximal tubules with all mitochondrial profiles shifted to large circular shapes with diminished cristae. There was loss of the plasma membranes of the lateral cellular interdigitations, but the brush border was maintained in many tubules. Measurements of mitochondrial size showed a marked increase of individual mitochondrial area from 0.38 ± 0.036 to 1.71 ± 0.78 μM^2 (SE ±; $p<0.0001$). There was marked interstitial fibrosis and tubular atrophy involving the subcapsular zone, medullary rays and the labyrinth. The liver pathology of mitochondrial enlargement (the basic abnormality in this patient) in MMA has been reported in mouse models and in a single human case, while the detailed renal pathology has not been described in humans.
Conclusions: With this study, we provide evidence that the renal mitochondropathy of MMA is characterized by megamitochondria formation in the proximal tubules of the kidney. Such changes may lead to tubulo-interstitial disease and have implications for treatments directed toward maximizing mitochondrial function in these patients.