and that the risk of classic KS is four-fold higher in patients with diabetes. There have been no comprehensive studies regarding patients with pure cirrhosis from Child–Pugh class A to C until to now.
Design: Cell counts and HHV-8 antibody and DNA were detected in blood samples from 108 cirrhotic patients without diabetes and 108 age- and sex-matched healthy controls.
Results: Mean lymphocyte, monocyte, and platelet counts were significantly lower, higher, and lower in Child-Pugh class C than class A cirrhotics (each $P < 0.02$), respectively. Monocyte counts were significantly greater in male and class B cirrhotics than female and class A or C cirrhotics (each $P < 0.05$), respectively. Hepatitis C virus (HCV)-infected cirrhotics had significantly lower lymphocyte and platelet counts than alcoholic patients and hepatitis B virus (HBV) infected or alcoholic patients (each $P < 0.05$), respectively. Seropositive rates and titers for HHV-8 antibodies were significantly greater in patients, particularly male and class B or C or HCV-infected cirrhotics, than the controls (each $P < 0.0001$). Seropositive female patients were significantly older than seropositive male patients ($P = 0.0130$), respectively. The rates and titers were also significantly greater in class B or C than class A cirrhotics (each $P < 0.05$). One HCV-infected male patient was positive for HHV-8 DNA (98 copies/mL).
Conclusions: Lymphopenia, monocytosis, and thrombocytopenia and seropositive rates and titers for HHV-8 antibodies were significantly greater in advanced versus mild cirrhotics. Monocytes increased, boosted, and then significantly decreased with Child-Pugh classes.

1627 Invasive Candidiasis Associated with Jejunal Ulceration and Perforation: An Under-Recognized Entity? Report of Three Cases

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Background: Candida species, a known colonizer of mucus membranes also has the ability to colonize and invade host cells and tissues to cause infection. The most susceptible patient groups include the immunocompromised, neutropenic patients, low birth weight infants and neonates with severe gastrointestinal diseases. More recent reports suggest association with certain diets and medications such as H2 blockers. A recent case of small bowel ulceration and perforation with invasive candidiasis prompted us to review previous cases of small bowel perforation at our institution.
Design: A restrospective review of all surgical cases of small bowel perforations received at Boston Medical Center from January 1, 2001 to September 30, 2011 was performed. Total of 44 cases were identified; the majority were secondary to trauma (43%), idiopathic causes (30%) and malignancy (9%). Less frequent causes were ischemic enteritis (5%), diverticulosis (5%) and foreign body (2%). Three cases of small bowel perforations (7%) were found associated with invasive *Candida* infection. Histological sections of the involved bowel segments, special stains (PAS and GMS), and microbiology culture results were reviewed.
Results: Histopathology of the segments of resected small bowel in all 3 patients showed invasive *Candida* enteritis with ulceration and perforation. All underwent exploratory laparotomy where perforation of the jejunum was found. The specific organisms subsequently grown in the abdominal drainage/peritoneal fluid sent for culture were *Candida albicans, Candida tropicalis* and *Candida glabrata* respectively, with all three organisms present in one case. No other etiology for the perforation was identified and none were immunosuppressed.

Table 1. Demographic and Clinical Presentation

Case	Age/ Gender	Associated Condition	Presentation	Site of Perforation
1	52/M	Gastrinoma	Acute abdominal pain	Jejunum
2	64/M	s/p Splenectomy 2° trauma	Small bowel obstruction	Jejunum
3	52/M	Liver Failure, Hepatitis C cirrhosis, Portal Vein Thrombosis, Anemia	GI bleed	Jejunum

M = male, s/p = status post, 2° = secondary, GI = gastrointestinal
Conclusions: Invasive Candida infection may be a primary cause of ulceration and perforation of the jejunum. Few cases have been reported in the literature but it is likely to be an under-recognized as well as a rare entity.

1628 H. pylori Infection Is Associated with DNA Damage of Lgr5-Positive Epithelial Stem Cells in Human Stomach

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Background: The G-protein coupled receptor Lgr5 is expressed in gastric antral epithelial stem cells and in cancer tissues. Mutagenesis of the epithelial stem cell genome has been proposed to underlie gastric cancer (GC) development. 8-hydroxydeoxyguanosine (8OHdG) is a DNA modification induced by reactive oxygen species that can be used to determine levels of DNA injury at the individual cell level. Studies have shown that H. pylori and associated inflammation are main GC risk factors, and this may be related to increased mutagenesis of the gastric epithelium. The aim of our study was to determine whether Lgr5-positive gastric epithelial stem cells are susceptible to mutagenesis associated with H. pylori infection.
Design: Lgr5 and 8OHdG expression was characterized in non-neoplastic gastric antral mucosa of gastrectomy specimens from 24 patients (16 with GC: 9 Hp positive (Hp+), 7 Hp negative (Hp-); and 8 without GC, all Hp-). To determine the extent of mutagenesis in Lgr5 positive epithelial cells (Lgr5+), expression of nuclear 8OHdG, a marker of DNA mutagenesis, was determined in co-stains with Lgr5 by immunofluorescence. Primary Lgr5 rabbit polyclonal and 8OHdG monoclonal mouse antibodies, followed by fluorescence labeled antibodies were used. Quantification of 8OHdG was done by spectral image analysis using a Nuance Trio microscope and CRI imaging software. In each case, at least 10 Lgr5+ and 10 Lgr5- cells from contiguous glandular cells were scanned.
Results: Overall the 8OHdG fluorescence maximum intensity (FMI) in Lgr5+ (mean 313.1, SD 143.1) was significantly higher than in Lgr5- cells (mean 286.5, SD 134.9) P=.03. In the group of GC cases in which both Hp+ and Hp- cases were available, the 8OHdG FMI (mean 263.6, SD 126.9) in Lgr5+ was significantly higher than in Lgr5- cells (mean 215.0, SD 106.5), P=.012 in Hp+ cases but not in Hp- cases (8OHdG FMI mean 342.8, SD 148.0 in Lgr5+ and mean 329.4, SD 134.8, in Lgr5- cells, P=.41).
Conclusions: These data suggest that DNA damage occurs in Lgr5+ gastric epithelial stem cells and that these cells may be more susceptible to oxidative stress than other gastric glandular cells. This is supported by the finding that 8OHdG accumulation was higher in Lgr5+ (stem) cells as compared to Lgr5- cells in patients with active H. pylori infection but not in those without H. pylori infection. These data support a role for H. pylori infection in mutagenesis of gastric stem cells which may underlie H. pylori associated gastric carcinogenesis.

1629 Clinical Significance of Isolated Cytomegalovirus Infected Intestinal Cells

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Background: Cytomegalovirus (CMV) infection of the intestinal tract is associated with high mortality in immunosuppressed patients. However, few studies have correlated peripheral whole blood CMV viral loads with histopathologic findings. Furthermore, there have been few studies determining the clinical significance of isolated CMV infected cells identified by hematoxylin & eosin staining and/or immunohistochemistry (IHC).
Design: We searched all non-consultation intestinal biopsies from 2006 to 2011 using our laboratory information system and further analyzed the cases with the diagnosis of CMV infected cells. We then selected as a control group thirty-one consecutive intestinal biopsy cases that had a negative CMV immunohistochemistry. The electronic medical record was reviewed for each case to determine peripheral blood CMV viral load detection by quantitative PCR, clinicopathologic features at time of diagnosis and clinical outcomes after discharge.
Results: Thirty-one patients with CMV positive intestinal biopsies confirmed by IHC were identified from 52% male and 48% female patients. The clinical setting of the 31 patients with CMV positive biopsies were solid organ transplantation (n=6), bone marrow transplantation (n=6), inflammatory bowel disease (n=6), ischemic colitis (n=6), chemotherapy for solid tumors (n=4), end-stage renal disease (n=3), and infection by Human Immunodeficiency Virus (n=1). CMV viral inclusions were identified by H&E stain in 26% (n=8) of cases, the remaining cases were identified by CMV IHC alone. CMV blood viral load was only positive in 16% (n=5) of cases. None of the negative control cases had positive blood viral loads. Seven cases that had only a single positive CMV infected cell; these cases had the following outcomes: worsening clinical symptoms that responded to antiviral therapy (n=3); did well without treatment (n=3); died after discharge to hospice without treatment (n=1).
Conclusions: CMV infection of the intestines is clinically significant, but will not always present with classic viral cytopathic changes. CMV IHC should be considered in any case where there is clinical suspicion. The identification of a single CMV infected cell by IHC should be regarded as clinically significant. Peripheral blood viral load has poor sensitivity in detecting CMV intestinal infection. However, future studies will investigate whether a positive viral load in patients with intestinal symptoms is predictive of intestinal CMV infection.

Informatics

1630 MeSH Term Trends in Pathology, a PubMED Survey of Articles in Pathology Journals

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Background: Medical Subject Headings are tagged for each journal article in NCBI database. We wanted to use MeSH terms to observe the alterations in research topics of pathology.
Design: 76 journals whose subject category were pathology in ISI Web of Knowledge Journal Citation Reports 2010 were included. ISSN numbers of these journals were used to download 223787 articles from PubMED in MEDLINE format. 2011 MeSH tree, journal and article properties in MEDLINE format were imported into Oracle database. Number of articles for each term was determined mimicking PubMED's explode function. To correct the numbers in regard to the increase in the publication numbers, term number was divided by article number to define a percentage. These percentages were used to compare the ratio between the last two 5-year-periods and named percent ratio.
Results: Although pathology journal articles constituted 1% of more than 21 million articles in PubMED, they covered 72.6% of total MeSH terms (26140). Last two 5-year were used to determine the recent changes in research fields; where >110% ratio was accepted as increase, <90% was decreased, others as relatively stable. In example, articles tagged with Molecular Diagnostic Techniques, Forensic Pathology, Surgical Pathology, Digestive System Neoplasms and Urogenital Neoplasms were increased whereas Immunohistochemistry and Telepathology were decreased.

Table 1

	# articles 2001-2005	# articles 2006-2010	Percent ratio
Autopsy	371	443	102
Histocytochemistry	9593	10095	90
Immunohistochemistry	11394	10750	81
Molecular Diagnostic Techniques	71	274	331
Pathology Department, Hospital	25	28	96
Forensic Pathology	40	317	680
Pathology, Clinical	305	361	102
Pathology, Molecular	0	56	N/A
Pathology, Surgical	207	330	137
Telepathology	64	57	76
Breast Neoplasms	1787	2024	97
Digestive System Neoplasms	2581	3660	122
Head and Neck Neoplasms	2039	2278	96
Nervous System Neoplasms	1355	1463	93
Soft Tissue Neoplasms	619	561	78
Urogenital Neoplasms	2256	4510	172

Percent ratios were corrected with article numbers; 34973 in 2001-2005 per 40759 in 2006-2010

MeSH terms introduced before and after 2000 were seperately analysed to figure out the changes.

Table 2

	# increased terms	# decreased terms	# stable terms
Terms introduced after 2000	828	808	81
Terms introduced before 2000	3878	6274	1304

Conclusions: We found NCBI databases useful for determining recent trends in pathology. Analyses including journals of other disciplines or selected MeSH terms can be used for searching the history as well as predicting the future research trends of pathology.

1631 Characterization of Tumor Heterogeneity Using High-Throughput Morphometric Assays (hTMA)

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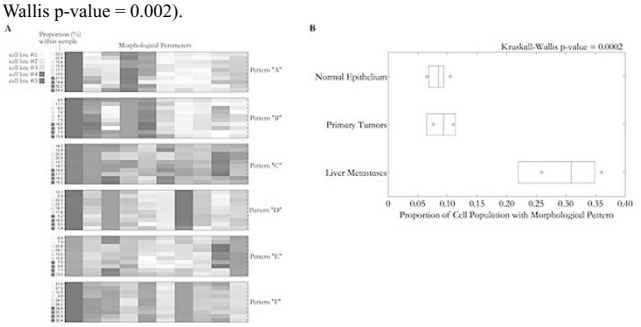
Background: The notion of tumor heterogeneity has received much attention. To attempt to better characterize this notion, we are developing high-throughput morphometric assays and supporting computational algorithms, which we believe will allow for better characterization of tumor heterogeneity and how this could be related to clinicopathological parameters such as tumor stage/grade, therapeutic response, and others.

Design: Various previously established pancreatic cell lines derived from both primary and metastatic components of pancreatic tumors were examined by digital microscopy. From these digital images, over 200 morphologic parameters per cell were extracted from a representative microscopic field from a given specimen. Subsequently, the resulting data were processed by a recently developed context specific clustering algorithm to reveal the distinct patterns of cellular morphology present and the distribution of these patterns within a given specimen.

Results: The analysis of nine cell lines derived from pancreatic tumors revealed that multiple distinct and consistently present cellular morphological phenotypes existed within the context of cell lines grown in the laboratory setting (Figure 1A). Interestingly, certain cellular morphological phenotypes were found to exist in consistently different portions when comparing cell lines derived from the metastatic component of pancreatic tumors as opposed to cell lines derived from the pancreatic tumors that did not exhibit metastatic disease clinically at the time of surgical resection (Figure 1B, Kruskall-Wallis p-value = 0.002).



Conclusions: The utilization of the proposed high throughput morphometric assay allowed for the examination of adherent cells and the identification of distinct cellular morphological patterns whose distributions were significantly associated to relevant clinicopathologic parameters. These preliminary results support the overall hypothesis of tumor heterogeneity and reveal that examination of this heterogeneity may have implications toward better understanding of tumor biology. Our future plans including extending this approach to the evaluation of histologic preparations of tissue specimens.

1632 An *In Silico* Approach to Finding the Expected Frequency of Coincidental Overlaps for *In Situ* Hybridization Using Dual-Colour Fusion Probes

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Background: *In situ* hybridization (ISH) is a commonly used technique, frequently used to assess for the presence of chromosomal translocations using two-colour fusion-type molecular probes. The success of the technique depends on the detection of (abnormal) signals not seen in normal cells. The coincidental overlapping of probes may mimic an abnormal fusion pattern, and it is generally distinguished from a true pathologic finding by employing statistical methods based on the frequency of these events in normal controls. However, the difference in nuclear size between normal cells and tumour cells may cause the cutoffs to be inaccurate for borderline cases.

Design: The frequency of overlap patterns was calculated using a spherical *in silico* model of the cell nucleus and random probe locations, using GNU Octave. The parameters in the model include (1) nuclear size (Dn), (2) the maximal distance between the centres of two signals in order to call an overlap in the image plane (res), and (3) orthogonal to the image plane (z_res), and (4) the number of nuclei to randomly generate. Factors (1), (2) and (3) were varied to understand their affect on frequency of overlaps and compared to experimental frequency data of normal controls scored manually at the microscope. A random distribution of the probes was assumed.

Results: The *in silico* model reproduces the frequency trends of overlaps in the experimental data, and furthermore, allows extrapolation to any given nuclear size. Larger nuclear size is associated with a decrease in overlaps The frequencies of common overlap patterns are functions of a non-dimensional number (res^2*z_res/Dn^3).

Conclusions: The *in silico* model demonstrates that in a tumour with large nuclei, the expected frequency of various overlap signal patterns is overestimated, if based on counts from smaller (normal) nuclei. This model can be used to determine the expected number of cells with coincidental overlaps for a tumour with any given average nuclear size. Employing criteria derived from this approach should increase the sensitivity of ISH tests uing dual-colour fusion probes.

1633 Implementation of a Web-Based Digtial Pathology Consutation Portal (DPCP) for Providing Second Opinion Consultation Services

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Background: UPMC has developed a web-based tool for digital pathology consultation. The tool allows outside entities to enter case data and upload files, including whole slide images. The internal tool allows our pathologists to view requests and submit diagnoses securely at their computers.

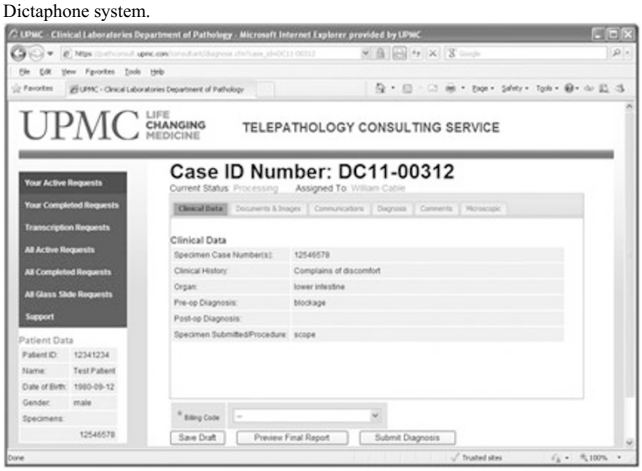
Design: Web tool features:

Clients: offers SSL (https) secure login to submit patient data, upload images, view status, and view and print second opinion reports.

Host Pathologist: hosts the diagnosis tool where pathologists can view whole slide images, annotate and capture image snapshots, view clinical data, and submit diagnosis.

Host Consultation Services: allows managers to triage requests, monitor workflow, and maintain personnel data.

Host Transcriptionist: contains data entry fields for diagnoses dictated through current Dictaphone system.



Technology: The web portal features an asynchronous uploader supporting transfers of multi-gigabyte whole slide image files. The slide viewer is an internally-developed Java applet, supporting multiple WSI formats with annotation capabilities. The application was coded in ColdFusion.

Results: We set out to develop a tool that could accept large image files that was both robust and format-agnostic. The submission tool needed to capture necessary clinical information for consultation while being reliable and user-friendly. Security measures needed to be implemented to protect patient privacy. A streamlined workflow needed to be developed to ensure prompt turn-around time, and buy-in by institute pathologists.
Conclusions: The digital telepathology solution allows clients to quickly access the expertise of our institute pathologists. Our institute has worked to overcome the significant challenges inherent in web-based telepathology, making this solution viable for second opinion consultations.

1634 Validation of Automated Image Analysis for Hematopathology

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Background: Whole slide image (WSI) analysis has great potential for standardizing diagnostic interpretation, streamlining workflow, and improving patient care. Automated differential counting of whole blood smears is one potential challenging application due to the size of the WSI files, inherent image artifact, and stain variability. There are also limitations contingent on properly selecting the relevant areas of interest. Our goal was to evaluate the effectiveness of an automated region of interest (ROI) algorithm by comparing it to manual ROI methods.
Design: Five randomly selected peripheral blood smears were scanned at 20x using an Aperio CS scanner. In the manual group (MG), the areas to be analyzed were selected by a trained user that visually selected high power snapshots from WSI of peripheral smears. In the automated group (AG), Definiens Developer XD was used to automatically detect the ROI and extract the white cells. MG and AG data served as independent data sets which were analyzed by a standardized MATLAB application. The application subclassified the white cells using linear discriminant analysis. All of the white cells were accurately classified by a hematopathologist to measure any differences in performance of the MATLAB application between the groups. The MATLAB application served as an objective benchmark of the inputs (MG and AG) with respect to the desired, clinically useful output (classification).
Results: 1919 cells were evaluated in the MG. 3190 cells were evaluated in the AG. The MATLAB classifier was most successful with the neutrophil cell line (98% accuracy for both MG and AG). The eosinophils had the lowest classification accuracy (67% for MG, and 72% for AG). There were no significant differences in percent classification between MG and AG for any of the cell types. Thus the algorithm to automatically select the ROI performed as well as a trained user who manually selected the ROI.
Conclusions: This novel technique using WSI to perform differential counts via automated techniques allows streamlining of workflow. The labor intensive portions of the differential count can be automated with subsequent review and fine tuning by the pathologist. Excellent concordance was achieved between manually selected ROI inputs and automatic ROI techniques.

1635 Whole Slide Imaging Telepathology (WSITP) for Primary Diagnosis in Surgical Pathology: A Comprehensive Validation Study at University Health Network (UHN)

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Background: UHN pathologists began using WSITP for making frozen section diagnosis in 2006. Given the success of this program, we are now considering using WSITP for routine primary diagnosis for surgical pathology cases originating at a partner hospital located 400 miles north of Toronto. 200-400 slides (25-40 cases) per day are sent from this facility to UHN for sub-specialty reporting. Prior to implementing WSITP, to avoid shipping these cases to UHN, there is an absolute need to ensure that the diagnostic performance of WSITP is at least equivalent to light microscopy (LM).
Design: As a practical approach to WSITP validation, we are determining whether the same pathologist can make the same diagnosis for cases reviewed initially by WSITP and then by LM. UHN pathologists, in 13 sub-specialty areas, prospectively select cases that represent what they encounter for primary diagnosis. Full cases are digitized using a scanner (Omnyx, LLC) and then reviewed using Omnyx viewing software. Preliminary diagnoses are recorded before the same pathologist reviews the cases by LM prior to final sign-out. There is no washout period between digital and LM review. The ability or inability to make a digital diagnosis along with diagnostic concordance between digital and LM are recorded. Discrepancies are considered major or minor, depending on their perceived impact on patient care.
Results: In the first 1.5 months of this 6-12 month study, 184 cases (> 1900 slides) from 8 sub-specialty areas have been reviewed. The cases range from small biopsies (1 slide with multiple levels) to large resection specimens (> 45 slides). Technical issues related to sub-optimal histology have prevented pathologists from making digital diagnoses in 2 cases and minor discrepancies have been noted in 3 cases. No major discrepancies have been observed. Complete agreement between digital and LM diagnosis has occurred in 179 cases (97%).
Conclusions: We have every indication that WSITP will be safe and accurate for making primary diagnoses on > 95% of cases originating at our northern partner site. It is likely that digital diagnoses would need to be deferred to glass slide review in only a small minority of cases. By no longer shipping all of these cases to UHN, WSITP will result in reduced transportation costs, removal of the risk of slides becoming lost or damaged during transport and reduced turnaround times with improved continuity of patient care.

1636 Breast Carcinoma Ki-67 Labeling Index: Comparison between Image Analysis and Expert Human Scoring with Discussion about Choosing Which Areas To Analyze

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Background: Proliferation is a key prognostic factor in estrogen receptor (ER) positive breast cancer, measured by counting Ki-67 staining. This is labor intensive and can be automated by image analysis. There are no well-established criteria for exactly how to assess Ki-67 in breast tumors with image analysis. This means that current image analysis systems must be closely supervised by their human operators, preventing significant automation of this task.
Design: 74 ER positive, resected breast tumors were stained with Ki-67 per vendor procedure (clone 30-9; Ventana, Tucson, AZ). Most-active areas were identified by an expert and then scored. This expert then scored the entire tumor section. Using highly-supervised methodology, four regions of interest (ROIs) from the most active areas were scored using an FDA-cleared image analysis system (VIAS; Ventana); then four additional ROIs were added to this analysis (the additional fields were selected from active-appearing areas elsewhere in the tumors). In a subset of 11 cases, additional scores were obtained by using random ROI selection. Statistical analyses were carried out (correlation coefficient).
Results: Image analysis correlated with the expert for both entire tumor and hot spot scoring (see Table 1). There was no significant difference in image analysis scores whether hot spots or entire tumors were analyzed, but in the 11-case subset a random ROI pattern did result in significantly lower scores (score difference with hot spot 9.18, 95%; confidence interval 5.44, 12.92). In almost all cases, manual override of automatic nuclei selection within an ROI was required. Also, the system had difficulty "seeing" negative staining nuclei even when these were manually included for analysis.

Correlation Coefficients (95% confidence intervals)

	hot spot (IA)	whole slide (IA)
human	0.882 (0.815, 0.925)	0.863 (0.788, 0.913)
hot spot (IA)	n/a	0.969 (0.951, 0.981)

IA: Image Analysis

Conclusions: There was good concordance between a supervised image analysis system and an expert human scorer. Although focusing on hot spots did not affect scores, random ROI selection did; therefore ROI selection can cause inter-observer variation of image analysis. Image analysis can over-estimate by selectively seeing positive nuclei, making manual supervision important. This appears to be an issue with most current systems. Future development should focus on better automation (less and/or more efficient supervision). Guidance of ROI selection should be a priority; it is only a matter of time before whole-section analysis becomes available.

1637 Co-Reporting of HPV and Pap Cytology Results: Informatics Experience at a Large Academic Women's Hospital

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Background: Human papillomavirus (HPV) DNA tests have been FDA-approved for co-testing in women 30 and older and as a reflex test for women with ASC abnormalities. Reporting Pap cytology diagnoses along with HPV test results in these circumstances has posed significant informatics challenges for cytology laboratories. The aim of this study is to describe our experience adding new functionality to our anatomic pathology laboratory information system (LIS) to provide this capability.
Design: Changes to our LIS (CoPath ver. 3.2, Cerner) and workflow were implemented in May 2005. Cytotechnologists select the HPV request type in the LIS during accessioning. Cases with pending or reflex HPV tests are tracked by the LIS, providing visual alerts to pathologists prior to sign-out. The LIS generates barcoded labels and a log of specimens designated for testing (Digene HC2). In the case of reflex testing, this occurs after the assignment of an ASC diagnosis by the pathologist. HPV test results are entered into the LIS by cytotechnologists using barcoded diagnostic quick-texts and combined with the cytology interpretation into a single report prior to sign-out. To evaluate the effect of the LIS changes we compared various parameters before and after implementation (2003-2010). Clinician satisfaction was solicited in an informal manner.
Results: Integration of HPV testing and reporting into the LIS has enabled cytotechnologists to order and perform testing under the supervision of microbiology staff, aided the interpretation of cases, and provided a shift in revenue to cytology. It has been particularly well received by our clinicians who desired a single consolidated report. HPV requests increased 60% over the time period. The LIS and related workflow changes had no significant effect on Pap turn-around time, but were associated with a 3% absolute increase in the number of ASC-US results. There was no significant change in pap volume, and no significant change in ASC-H or other abnormal results.
Conclusions: Modifications to our LIS functionality were required to handle new demands for HPV testing. LIS-generated barcodes, logs, and alerts helped automate the composition of combined reports. After implementation, there was an increase in ASC-US diagnoses, but this may have been due to a new laboratory policy of targeted educational re-screening of negative Pap tests preceding all histopathologic CIN2+ diagnoses also begun in 2005. There was no delay in turn-around-time despite a significant increase in HPV test volume.

1638 Telecytology as a Tool To Screen Cervicovaginal Smears

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Background: Telepathology is rapidly becoming a part of routine practice. This technology is necessary to provide quality care, particularly for patients with limited access to medical care. The current study evaluates the application of this technology to screen cervicovaginal smears.
Design: 52 consecutive routine cervicovaginal smears were reviewed by a cytotechnologist, on a light microscope. These 52 cases were reviewed by the same cytotechnologist using a telepathology system comprised of a Nikon Eclipse 55i microscope and a Nikon DS-L2 digital camera. The slides were driven from a separate site by a medical student. The areas of interest and time spent on the slides were directed by the cytotechnologist on the computer screen. The results were noted for diagnoses

(utilizing Betheseda system), concordance, and time taken for slide review. The study was retrospective, to avoid unnecessary delays in turn-around time.
Results: 47 cases (90.4%) screened via telepathology were concordant with the diagnoses made on the microscope. Of the 5 discrepant cases: 3 cases- appeared to have scant material on computer screen either due to excess blood or atrophic smear. Case 4: cytoplasmic fragments misinterpreted as *Trichomonas vaginalis*. Case 5: ASCUS diagnosed as AGUS. The average screening time: 5.27 ± 1.05 minutes on microscope and 6.02 ± 1.50 minutes remotely.
Conclusions: The difference in average screening time between the microscope and the computer screen was minimal (45 seconds). 2 of the 5 discordant cases pose potential problems for patient management: ASCUS and AGUS are managed differently; diagnosis of trichomoniasis, besides treatment, has significant social implications. The remaining 3 discordant cases would have required repeat smears but caused no harm in terms of management. Of note, the discordant cases were not reviewed by pathologist, which might have reduced discordance rate.
Our preliminary study (concordance rate 90%) is very encouraging. The reported rate of intra-observer agreement by light microscopy is approximately 78% and 58% for telecytology. In view of such wide variability, our study suggests real potential for implementing the technology for screening programs in remote areas. This study also indicates that the microscope can be operated effectively by a non-pathology trained person and the smears can be read on computer screen without prior training. With training the accuracy of diagnosis would improve. Additional study is needed to assess reproducibility and further assess intra-observer variability.

1639 Contextual Inquiry of Air Force Medical Service (AFMS) Pathology: Identify the Unique Needs and Work Practices Prior to Implementing a Digital Pathology System

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Background: Recent advances in whole slide imaging are initiating a move towards wide spread adoption and implementation of digital pathology (DP) in anatomic pathology practices. The Air Force Medical Service (AFMS) is exploring efforts to implement DP into its pathology practice. Prior to designing a new DP system, the unique needs and preferences of AFMS pathologists and their pathology system should be identified. Contextual Inquiry (CI) is a social tool for understanding and capturing in-detail aspects of work from the perspective of the person performing the work, and is used to help identify the worker's needs. CI is commonly used by software developers, and was previously used to help design other hospital information systems.
Design: A research team composed of an academic pathologist and 3-5 trained observers interviewed pathology/histology personnel at 3 AFMS pathology labs following CI guidelines by Holtzblatt et al. Notes representing user-provided data from all sites were documented during the observation sessions. These notes were arranged into an affinity diagram, a hierarchal organization of the notes based on common themes in the data. Five models were created to help visualize the data: sequence, flow, artifact, physical, and cultural models.
Results: A total of 22 pathologists, 4 residents and 7 histotechs were observed. A total of 1132 affinity notes were documented. Notes were generalized into 74 statements and grouped into 27 second level, 9 first level and finally 5 main level categories--Workflow and Workload Distribution, Quality, Communication, Military Culture, and Technology. AFMS pathologists work flow needs were similar to civilian pathologists, including need to deliver accurate diagnosis in a timely manner, conduct quality assurance, consult with other pathologists, communicate effectively with clinicians, and track and manage cases. Unlike their civilian counterparts, AFMS pathology system has unique staffing limitations and need to serve a large patient population distributed across the globe.
Conclusions: Based on findings, AFMS pathology has more to gain from DP and is better positioned to implement DP than civilian pathology practices.

1640 Telecytology for Rapid Preliminary Diagnosis of Ultrasound-Guided Fine Needle Aspiration of Axillary Lymph Nodes in Patients with Prior History of Breast Carcinoma

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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. However, there is a need for organ based and body site specific studies on use of telecytology for immediate assessment of FNA to evaluate its pitfalls and limitations. We present our experience with use of telecytology for on-site evaluation of ultrasound guided fine needle aspiration USG-FNA of axillary lymph nodes.
Design: 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 by a cytotechnologist to a pathologist who rendered preliminary diagnosis while communicating with the on-site cytotechnologist over the Vocera system. The accuracy of preliminary diagnosis was compared with final diagnosis, retrospectively. Kappa statistic was used to compare agreements between telecytology preliminary diagnoses versus final diagnoses.
Results: A total of 19 female patients (mean age 50.5 yr.) with prior history of breast carcinoma underwent USG-FNA of 23 axillary nodes. Preliminary diagnoses of benign, suspicious/ malignant and unsatisfactory were 35%, 52%, and 13% respectively. Only one of the 8 cases that were initially interpreted as benign was reclassified as suspicious on final cytologic diagnosis. Eleven of 12 suspicious/malignant cases on initial cytology corresponded with a malignant diagnosis on final cytology. One suspicious case was reclassified as benign on final cytologic diagnosis. All unsatisfactory cases remained inadequate for final cytologic interpretation.There was excellent agreement between telecytology and final cytologic evaluation (Kappa value 87%). Presence of additional material on Pap stained slide and cell block was the main reason for discrepancy, accounting for the two discrepant cases.
Conclusions: This retrospective study demonstrates that on-site telecytology evaluation of USG-FNA of axillary lymph nodes in patients with prior history of breast carcinoma is highly accurate compared with final cytologic evaluation. It allows pathologists to use their time more efficiently and makes on-site evaluation at a remote site possible.

1641 Quantitative Evaluation of the Morphological Heterogeneity in Breast Cancer Progression

MC Lloyd, K Rejniak, JO Johnson, R Gillies, R Gatenby, MM Bui. Moffitt Cancer Center, Tampa, FL.
Background: Cancer cell heterogeneity is known as a factor of cancer progression and resistance to therapy. Quantitative evaluation of tumor heterogeneity have been carried out at the molecular and genetic scale; however, little is known at the cell morphology scale regarding to the variability of individual cells with respect to phenotypic core traits (proliferation, survival, morphology, and metabolism). We hypothesize that while genetics and signaling networks are the basis of core traits, cells' ability to perform core trait functions under diverse conditions within the physical microenvironment may decide their trends in tumor growth dynamics. This study is to investigate the feasibility of computational morphological analysis algorithms to predict the breast cancer progression.
Design: Multiple serial sections (4 μm) of 12 lobular and ductal breast carcinoma resection were collected retrospectively. Digitalized HE and IHC slides (10 biomarkers) were computationally investigate the morphological features of cancer cells on the whole digitalized slides. Sophisticated computer algorithms were developed to segment cancerous regions from the tumor's microenvironment and other non-malignant tissue structures. Every cancer cell was also segmented individually (~1.5-3million/sample depending on the tumor size) and 5 morphological features were extracted from each cell (nuclear size, N:C, nuclear intensity, cyto intensity and Haralick texture). This expansive data set was parsed and interrogated using co-variant analyses to indicate if subpopulations of cells at the leading edge of invasive cancers were morphologically identifiable.
Results: A single morphological parameter was helpful in identifying subpopulaitons of cells spatially related to the invasive edge of GIII tumors, while not significantly present in GI samples. A multiparametric analysis of morphological features and molecular expression in the same sample indicated 2.6% (n=264) of the total cancer cell population within 250μm of the invasive edge of GI tumors, 14.7% (n=1,338) in GII, and 21.7% (n=19,584) in GIII. This represented GII>GI (5fold) and GIII>GII (15fold) in identifiable subpopulations of aggressive cells.
Conclusions: Morphological heterogeneity of breast cancer can be quantitatively evaluated by image analysis and are correlated with tumor progressiveness to aid in treatment descision making.

1642 Quantitative Evaluation of Histologic Grade of Breast Cancer Using Digital Image Analysis

MC Lloyd, JO Johnson, J House, E Henderson, D Tacha, MM Bui. H. Lee Moffitt Cancer Center, Tampa, FL; University of South Florida, Tampa, FL; Biocare Medical, Concord, CA; Moffitt Cancer Center, Tampa, FL.
Background: The Nottingham combined histologic grading system is recommended to grade invasive breast carcinoma. This semi-quantitative evaluation is read by a pathologist using a standard formula. Intra-pathologist variability has been reported. Digital pathology and algorithm development offers the ability to expand the pathologist's toolbox in order to enhance histological diagnosis. This study will investigate the feasibility and reproducibility of quantitative histologic grading of breast cancer.
Design: Twenty-five retrospective cases of ductal and lobular carcinomas of various Nottingham grades were randomly selected. H&E slides were blinded and digitized using a slide scanning instrument. Two pathologists diagnosed and scored each case. Data recorded included Nottingham score for tubule formation, nuclear pleomorphism and mitotic count, combined Nottingham score and grade. Simultaneously, the digital images were used to create a customized image analysis algorithm by using the digital slide to isolate tumor regions via pattern recognition and to score the patterns for each Nottingham scores. The computationally derived scores were compared back to the pathologist's scores. Furthermore, five cases were blindly scored in duplicate to investigate intra-pathologist and intra-image analysis variability.
Results: There was heterogeneity of scoring between pathologists (12/25 cases). The inconsistencies mostly seen in GII cases and was <2 Nottingham scores. Although each pathologist scored 5 cases in duplicate in the same sitting, variability was observed (3/10), one of which resulted in a grade change from II to III. The computer, however, scored all samples with less heterogeneity (<1 Nottingham score) and was 100% consistent in duplicate samples. Quantitative PHH3 based mitotic score indicates a difference between Nottingham grades [GIII 187 mitotic figures in $5mm^2$ vs 9 in GI].
Conclusions: The development of an image analysis algorithm to quantitatively evaluate histologic grade of breast cancer is feasible and reproducible. This algorithm is not intended to replace the expertise of the pathologist, but to be used as an adjunct tool to enhance the diagnosis. Additionally, PHH3 is being used to molecularly identify mitotic figures. Therefore with further evaluation, more quantifiable scoring methods of mitosis are anticipated to upgrade Nottingham score.

1643 Using Computerized Simulations To Measure the Effects of Full Adoption Whole Slide Imaging on Medium and High Volume Clinical Histology Laboratories

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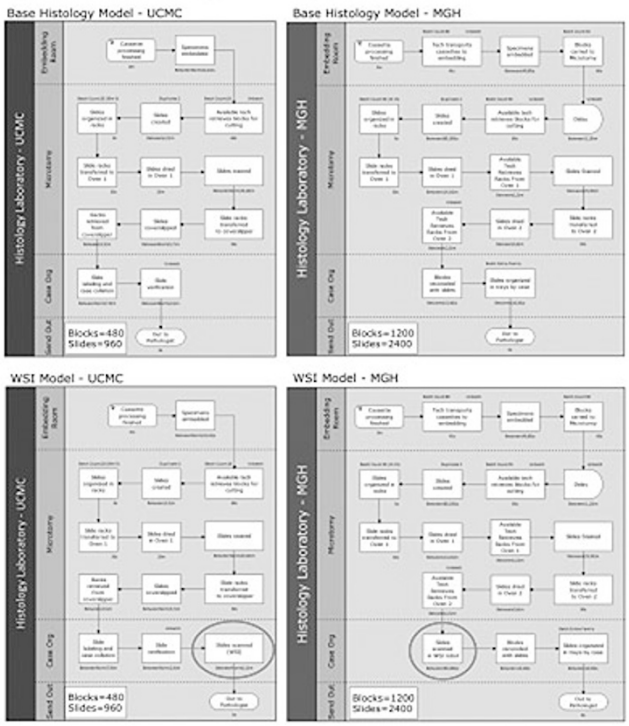
Background: Whole slide imaging (WSI) has been touted as the future of anatomic pathology, with estimates of full adoption (scanning all slides from the histology laboratory) occurring sometime in the next 5-10 years. While WSI technology has improved greatly (scan times of 60 sec for a 15x15 mm^2 area at 400X), there has been little consideration of the effects WSI may have on the current workflow of the clinical histology laboratory.

Design: Workflow process data for the clinical histology laboratories at the University of Chicago Medical Center (UCMC, medium volume lab) and Massachusetts General Hospital (MGH, high volume lab) was collected in regards to workflow process steps, equipment and personnel resources, and average daily volume of routine assets (blocks and slides). Histology lab workflow process models were created with business process analysis software and simulations run without, and then with, the presence of one or more WSI devices.

Results: The clinical histology laboratory models upon which simulations were run are shown in Figure 1 (red circles = WSI devices).

Figure 1: Clinical Histology Laboratory Process Models, MGH and UCMC



Simulation data showing histology lab turn-around-time (TAT) for a full day with all routine assets processed are shown in Figure 2. Adding WSI to the workflow of both the medium and high volume labs (60-180 sec average scan time per slide) demonstrates a dramatic increase in daily TAT.

Figure 2: Clinical Histology Laboratory Process Turn-around-time (TAT), 1 days work

UCMC: 480 blocks, 960 slides

# WSI Devices	0	1	2	3	4	5	6	7
Histology TAT (hrs)	8.2	40.8	24.6	12.3	9.6	8.4	8.4	8.4

MGH: 1200 blocks, 2400 slides

# WSI Devices	0	1	2	3	4	5	6	7	8	9	10	11
Histology TAT (hrs)	10.4	108.0	54.6	34.6	27.9	17.4	14.7	12.8	11.3	10.5	10.4	10.4

Conclusions: Full adoption of WSI is unfeasible today without either 1) making drastic changes to the current workflow of the histology laboratory or 2) adding many WSI devices (5-7 for a medium volume lab, 9-11 for a large volume lab). Given the space and cost constraints of having more than 2-3 WSI devices in most histology laboratories, future efforts should be placed in redesigning and optimizing the clinical histology workflow in preparation for full adoption of WSI.

1644 False Discovery and Fairy Tales in Gene Expression Analysis

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Background: Analysis of gene expression data can be misleading due to overfitting. Genes are highly connected in cellular networks with frequent duplication across pathways. We hypothesize that as more genes are determined to be relevant to a disease outcome, it is more likely any two genes are connected in a known pathway. Cancer is a heavily studied disease area. We also hypothesize that the likelihood of discovering a gene to be a member of a pathway for which at least one member has been studied in association with cancer is high.

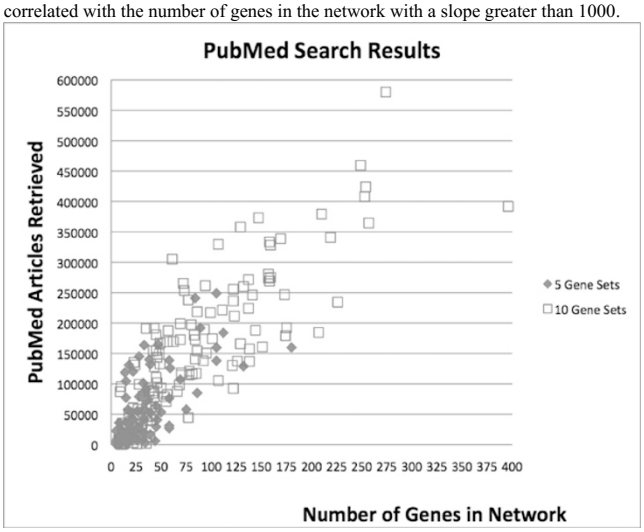
Design: A master set of 12000 genes was constructed using an Affymetrix microarray probe set. One hundred sets of five and ten genes each were created by random selection from the master set. Dykstra's algorithm was used to build pathways using MetaCore, a commercial database of gene and protein interactions, using the five and ten gene sets as inputs. We measured the frequency with which pathways could be generated using one, two and three maximum intervening steps between any two genes included as input. When networks were generated, we constructed a Boolean statement using the "or" operator to join the network genes and the "and" operator to join the gene set with the term "cancer". This Boolean construct was used to search Pubmed for publications relevant to cancer for any member of the network.

Results: Frequency analysis is summarized below.

Table 1 Pathway Generatino Frequency

Maximum intervening steps allowed	1	2	3
5-gene input	0%	28%	67%
10-gene input	6%	70%	96%

The number of publications retrieved using the Boolean search strings was linearly correlated with the number of genes in the network with a slope greater than 1000.



Conclusions: Given the connectivity of genes and the proliferation of literature on cancer, using pathways with relevant literature on cancer to support mechanisms elucidated from gene expression data analysis can be misleading. In particular, techniques that generate many hundreds of genes are more likely to result in higher false discovery rates. This can create problems when using pathways databases and literature on cancer to develop relevant mechanisms to support gene discovery. Literature support or network connectivity cannot be used in isolation to support hypotheses generated from analysis of gene expression data.

1645 PathRez – A Concept Based Relational Database Model for Archival and Retrieval of Static Images and Virtual Slides

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Background: Online image libraries are a valuable resource for trainees and practicing pathologists. Whole slide images (WSI) have added a new dimension to them. Several online libraries are available, but many lack indexing to allow easy searches. The result is an unsatisfactory user experience due to inefficient process of image searches. Our study was aimed to build a concept based relational database model to optimizing image and WSI archiving and retrieval.

Design: PathRez, a web interfaced and indexed SQL server 2008 (Microsoft, Richmond, VA, US) database of images and WSI, was built using the .NET framework 4 platform (Microsoft, Richmond, VA, US). HTML, CSS and JavaScript was used for client side scripting and VB.NET was used for server side coding. Based on anatomic location, most recent accepted pathologic classification of disease entities, basic pathologic process and image attributes, a 4-tier disease concept model was developed which consisted of a set of unique numerical codes (concept ID). An image or a WSI was represented in the database by one concept ID. Each image also was accompanied by the following fields; Diagnosis and comment. This classification was used for optimizing database search and to render the web user interface at runtime. The user interface consisted of a header, navigation bar and a content area. Images were displayed using an in-built image viewer or vendor-provided viewers for WSI. The website is currently hosted in the UPMC system and is accessible from work and home (via remote access).

Results: The web interface welcomes the user with a random image from the databank, which currently contains over 500 WSIs of genitourinary, head and neck, pulmonary, infectious disease and cardiac pathology and 500 gross and microscopic images. A brief description with the contributor name of accompanies the latter. Image can be searched using quick search function or advanced search features.

Conclusions: This concept based image library provides a reference database for users at different stages of pathology training and practice. The underlying relational database design makes this a unique tool for fast and efficient searches. Such a tool is applicable as an online reference for beginners, instructional resource for tutors, trainee evaluation tool and a forum for sharing interesting images.

1646 Electronic Body Management Database Application (*e*-BDF): Implementing a Unique Web-Based Program with Impact on Work Flow and Error Reduction

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Background: Final body management is a complex process requiring coordination among hospital departments, medical examiner (ME), funeral home, gift of life (GoL), and legal next-of-kin (NoK). Commonly a paper form documents and tracks body management, and was supplemented by a Microsoft ® Access database. However, limitations included entry duplication, illegible information, lack of instant verification, and no real-time updates or archival verification of body status.

Design: A web based *e*-BDF application was created by our Application Development Technology Team (ADTT), with collaboration by pathology, nursing, GoL, security, and ME. It was written in C# on the .NET 2.0 platform, with data stored on a Microsoft ® SQL server. Users were assigned various designations. Application entry includes; death notifications, body delivery status/pick-up, autopsy status, and archived information. The e-BDF is initiated by entering the deceased MRN by a registered nurse (RN), under death notification. The e-BDF is divided into six tabs documenting patient personal information, legal NoK, autopsy, GoL, ME, and final checklist. Each tab offers guidance with real time help plus phone contact. An autopsy request requires a physician to discuss/obtain permission from the legal NoK, and document the reason. A preliminary *e*-BDF can be generated, facilitating body transport to the morgue. Later, a completed form is printed with signatures, by RN, physician, and NoK. The record is locked and archived with a history log. Each subsequent step is also documented, including morgue delivery, autopsy completion, and release by security. A hospital-wide training and annual recertification is required for users.

Results: The system has been in place for one year with the following benefits: eliminates paper forms, data entry duplication, and multiple hands off, reduction in data entry time by 50%, single system archiving, expedites GoL, ME, and funeral home processing, and 24/7 real-time access and updates. Furthermore, incomplete forms and error rates have almost become non-existent.

Conclusions: The *e*-BDF web-based application has significantly improved work flow with enhanced patient service, safety, and security. Furthermore, interdepartmental communication is improved with increased compliance to hospital policies for autopsy request and correct documentation of NoK. We continue to monitor and update the system as feedback is received.

1647 Diagnosing Adenocarcinoma of the Prostate by Computer Vision Methods

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Background: Based on our successful application of computer vision methods to detect endometrial carcinoma, we extend our analysis to acinar-type adenocarcinoma of the prostate, using region covariance descriptors in a dictionary-learning modeling framework and discriminative dictionary learning.

Design: We used 60 images of H&E-stained sections of radical prostatectomy specimens from 3 patients, 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. The annotated regions are then broken down into overlapping blocks of 200 x 200 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 $[I, I_x, I_y, \sqrt{(I_x^2 + I_y^2)}]$ features, giving rise to 8x8 covariance descriptors. The covariance descriptors from each class (cancer vs. benign) were used to learn a concise dictionary model. The experiments were repeated for varying dictionary size K and sparsity level T. Each test block was classified as being cancerous or benign based on which dictionary model gave the least representation error. 10-fold cross-validation was run on the entire dataset.

In discriminative dictionary learning, we vectorize $\sqrt{n}$ x $\sqrt{n}$ image patches into n-vectors, and a representative dictionary is learned for vectors from each class, under the constraint that each vector can be reconstructed by a sparse subset of the dictionary. 32 x 32 patches were extracted from images of each class and discriminative dictionaries were learned using 12000 labeled training patches, followed by three-fold cross-validation.

Results: Using region covariance descriptors, the overall classification accuracy of the image blocks into cancerous vs. benign regions was 88.24%. On average, we obtained a true positive rate of 98.3% and a false positive rate of 21.8%, with a standard deviation of < 1%. These results were obtained for dictionary size of K = 1600 and sparsity of T = 4, which were selected by cross-validation. In contrast, discriminative dictionary learning, which had performed well in detecting endometrial carcinoma, did not produce sufficient discrimination between the benign and cancerous regions in the prostate images, with classification accuracy close to a random classifier (~50%).

Conclusions: Computer vision is highly effective in diagnosing adenocarcinoma of the prostate. As different types of cancer have very different image characteristics, custom classification approaches should be developed for individual tumors.

1648 Histopathologic Correlation with Spectral-Domain Optical Coherence Tomography (SD-OCT), a Novel Digital Imaging Technique

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Background: Spectral-Domain optical coherence tomography (SD-OCT) is a novel 3D imaging technique that can produce images that resemble low-to-medium magnification photomicrographs directly from tissue, without glass microscope slides. In addition to potentially decreasing turn-around time for pathologic diagnosis, such 3D imaging is an opportunity to augment current histopathology knowledge. This project is an effort to characterize the 3D histopathology of vulvar Paget's disease using spectral-domain OCT.

Design: SD-OCT images were acquired from formalin-fixed, paraffin-embedded tissue blocks of vulvar Paget's disease (Bioptigen, Research Triangle, North Carolina, USA). 2 mm deep volumes of tissue were sampled with 500 x 500 x 1024 voxel resolution over small areas of the block (transverse resolution 20 microns, axial resolution 2 microns). OCT images underwent post-processing then were correlated with routine H&E stained slides. Virtual slices and 3D reconstructions were created.

Results: Low-magnification features were recognizable including nests of Paget's cells. Overlying hyperkeratosis, epidermal proliferation and dermis are all distinctly visible in the OCT images. Initial OCT images lacked resolution and contrast relative to traditional microscopy, but the image content suggests that additional features of interest are present and may be revealed with improved imaging technique and post-processing experience.

Conclusions: Rapid or near-instant diagnosis (either in vivo or in vitro) is a tremendous opportunity for pathologists. It is also a challenge, in that existing histopathology expertise must be expanded to include 3D information. As with previous SD-OCT images in pathology, resolution and contrast are less than with standard microscopy—this is being addressed with improvements to imaging technique and with new OCT systems that are optimized for pathology specimens. Finally, existing images will be useful in creating computer-assisted diagnosis systems.

1649 The Impact of Digital Pathology on Pathologists' Time

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Background: One of the factors that organizations must consider when evaluating the implementation of digital pathology is the impact to their pathologists' time. Stratman, Drogowski, and Ho presented results of time and motion studies performed in 2010 that quantified the time spent by pathologists on tasks related to routine case work. These tasks fell into three categories: workflow (case management), slide review (case analysis), and reporting (case documentation). Of the pathologists studied, it was found that a digital pathology solution has the potential to save an average of more than 13.4% of total case work time by eliminating case management activities. A follow-on question is understanding the difference in time spent by pathologists on slide review tasks when using glass slides versus digital images.

Design: This study utilizes two sets of cases. Each pathologist participant reads one set using glass slides and one set using digital images. After a washout period, each pathologist reads both sets again using the alternate method. The time spent reviewing the slide are compared within each pathology within each set between the two reading modes. This eliminates the bias of interpathologist timing differences and differences in difficulty between sets. Also, memory bias from repeated reads of the same case can be identified.

Results: The table below summarizes the results of slide review timings captured:

Summary of Time per Slide by Reading Mode

Pathologist	Slide Set	1st Read: Mode	1st Read:: # of Slides	1st Read: Mean Time per Slide (sec)	2nd Read: Mode	2nd Read: # of Slides	2nd Read: Mean Time per Slide (sec)
1	A	Glass	66	20	Digital	63	21
1	B	Digital	56	22	Glass	59	17
2	A	Digital	85	53	Glass	77	43
2	B	Glass	80	49	Digital	83	44

Pathologist 1 had moderate prior experience with the digital pathology application utilized in the study. Pathologist 2 had no prior experience with the digital pathology application utilized in the study. T-tests did not display a significant difference in mean time per slide between glass and digital reads.

Conclusions: Digital pathology has to the potential to be as efficient for reviewing slides as the microscope after a minimal training timeperiod. Provided that reporting methods and related tasks remain the same, the results of this study on slide review time, combined with those previously presented on savings in case management time, display a significant value-add opportunity for digital pathology implementation.

1650 Whole Slide Imaging System Resolution Evaluation Slide Set Development: Toward Standardization

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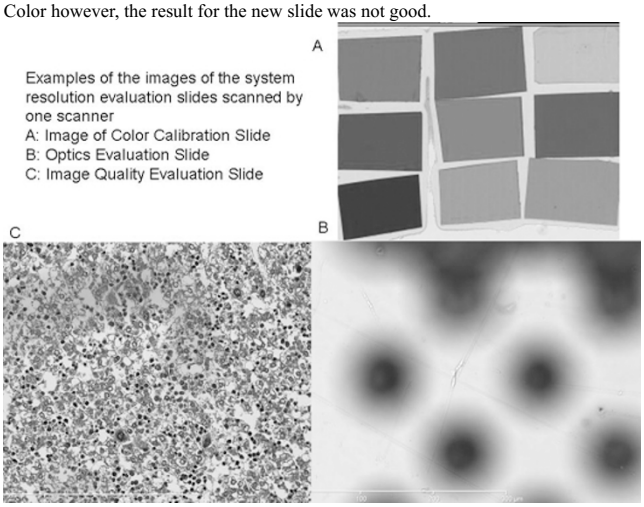
Background: System resolution of Whole Slide Imaging (WSI) system is very important toward standardization. While the most common reason for the variations of color and image quality is the variance in the protocols and practices in the histology lab, the image displayed can also be affected by variation in capture parameters, image processing and display factors in the digital systems themselves. It is difficult to identify which exactly causes the problem. We have developed the methodology to evaluate the balance as a system and identify the cause of the problem of image quality, color and system resolution. In addition to *the* two *calibration* slides *we have developed* for color and image quality, we *developed another* slide to evaluate optics of the WSI.

Design: In addition to the two calibration slides we have, we developed a slide to evaluate the optics of whole slide imaging system. Using the two calibration slides we can evaluate and standardize whole slide imaging but we can not identify exact cause of the quality issue in the system. These three slides were scanned by several different manufactures' scanner. From the corresponding whole slide images of the two calibration slides we previously developed we evaluated the color characteristics of the scanner and the image quality of the whole slide images each scanner produced. On the other hand, from the scanned image of the new slide, we evaluated the balance between optics, electronics and software and determined the cause of image quality degradation and color variations.

Results: The usefulness of the color and image quality evaluation slides was demonstrated. Furthermore, the new slide for Optics evaluation showed interesting results. Some system showed very good results in the Image Quality Evaluation and Color however, the result for the new slide was not good.



After the investigation of the system's component, we found that the system has relatively lower optics quality than image acquisition device If optics quality is improved, it is expected that the system resolution would also improve.

Conclusions: The importance of the additional slide to evaluate the optics of WSI from many aspects was demonstrated. The new slide could help improve system resolution of Whole Slide Imaging System toward Standardization.

Kidney

1651 Arhgap24 Downregulation Is Associated with Foot Process Effacement in Minimal Change Disease

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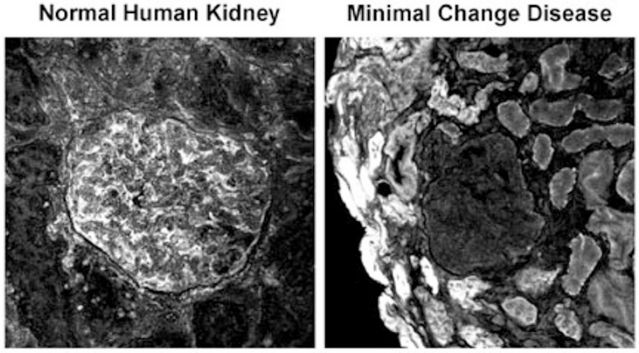
Background: In vitro studies on glomerular epithelial cells (podocytes) suggest that increased membrane motility may contribute to foot process effacement and proteinuria. Recently, we identified Arhgap24, as a gene that suppresses membrane dynamics of podocytes in vitro. In human and murine kidney, podocytes normally express high levels of Arhgap24 protein, but the protein is susceptible to degradation. Therefore, we hypothesized that Arhgap24 levels would be reduced in proteinuric kidney diseases such as Minimal Change Disease (MCD) where foot process effacement is the key diagnostic feature.

Design: We developed new antibody reagents to stain for Arhgap24 and validated their specificity. Using these antibodies, in a proof of concept study, we stained fresh frozen normal kidney and biopsy material from a 10 year old boy with MCD (proven by electron microscopy). We examined the immunofluorescent staining intensity of Arhgap24 in podocytes in both samples.

Results: 1) Our newly developed antibodies stain Arhgap24 in human podocytes in fresh frozen kidney tissue (Figure 1, left panel).

2) Arhgap24 levels are decreased in a patient with minimal change disease (Figure 1, right panel).



Conclusions: Our cell biological studies predicted that Arhgap24 downregulation would increase podocyte membrane motility in vitro and cause foot process effacement in vivo. Here we show, in a pilot study, that Arhgap24 expression is indeed decreased in a patient with diagnosed MCD, the prototypic kidney disease with foot process effacement. We are currently extending this study to additional patients and will also test the antibody reagents on FFPE kidney tissue. If successful, Arhgap24 downregulation would be a useful IHC marker of MCD when electron microscopy is not available. This study also shows how understanding of the cell biology of cultured podocytes can lead to a useful prediction of biologic behavior in patients.

1652 Renal Extramedullary Hematopoiesis Mimicking Tubulointerstitial Nephritis

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Background: Extramedullary hematopoiesis (EMH) is the presence and growth of hematopoietic tissue outside of the bone marrow, usually involving the liver and spleen. EMH of the kidney has rarely been reported. This is the first series outlining the clinicopathologic spectrum of intrarenal EMH.

Design: Patients (pts) with EMH in the kidney were identified from a biopsy database. Presenting clinical and laboratory findings and treatment and follow-up data were obtained. Pathology material was reviewed.

Results: Eight pts with intrarenal EMH were identified. The mean age at presentation was 68 yr (range 47-87); males predominated (M:F=7:1). All pts presented with renal failure, 2 with acute renal failure. The mean serum creatinine (SCr) at biopsy was 3.3 mg/dl (range 1.3-7.3). Six pts had proteinuria, 3 with nephrotic-range proteinuria. All biopsies showed a diffuse interstitial infiltrate that included megakaryocytes, granulocyte precursors (including many eosinophils) and clustered erythroid precursors, highlighted by immunostains for CD61, myeloperoxidase and glycophorin A. Tubular injury was present, but tubulitis was absent or rare. Two biopsies showed extrarenal extension. Two biopsies were misdiagnosed as tubulointerstitial nephritis (TIN). Five biopsies showed concurrent glomerular disease: 1 with fibrillary glomerulonephritis (GN) alone, 1 with fibrillary GN and chronic thrombotic microangiopathy (TMA), 1 with diabetic glomerulosclerosis and chronic TMA, and 2 with focal segmental glomerulosclerosis. All 8 pts had an underlying hematologic malignancy: 5, primary myelofibrosis; 2, an unclassifiable chronic myeloproliferative neoplasm (MPN); and 1, multiple myeloma (MM) with extensive bone marrow involvement. In 6 pts, the hematologic malignancy was diagnosed prior to renal biopsy by a mean of 78 mos (range 7-180); in 2 pts, renal EMH was the first manifestation of MPN or MM. Six pts also had EMH in the liver and/or spleen.

Clinical follow-up data were available in all pts, at a mean of 9.6 mos post-biopsy (range 1-23). Five pts were treated with hydroxyurea and/or other chemotherapy, 1 was treated with steroids, and treatment data were not available for 2 patients. The mean SCr at follow-up in 7 pts without end-stage renal disease (ESRD) was 1.8 mg/dl (range, 1.1-2.7). SCr improved in 2 pts to near-baseline; 5 had persistent renal dysfunction; and 1 progressed to ESRD within 3 mos.

Conclusions: EMH should be considered in the differential diagnosis of TIN, even in pts without a previously known hematologic malignancy. Intrarenal EMH may have associated glomerular disease. Treatment of the underlying malignancy may improve renal function.

1653 Membranous Glomerulonephritis Secondary to IgG4-Related Disease

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Background: IgG4-related disease (IgG4-RD) is a multiorgan inflammatory disease. Renal involvement is usually recognized in the form of IgG4-related tubulointerstitial nephritis (IgG4-TIN). There are case reports of glomerular disease associated with IgG4-RD, usually membranous glomerulonephritis (MGN). This is the first series of MGN associated with IgG4-RD.

Design: Patients(pts) were identified from a biopsy database based on the combination of MGN and clinical or histopathologic evidence of IgG4-TIN or other organ involvement by IgG4-RD. Presenting clinical data and treatment and follow-up data were obtained. Pathology material was reviewed.

Results: Eight IgG4-RD pts with MGN were identified. All pts had nephrotic-range proteinuria (ave 9.6 g/day;range 3.5-16). The mean serum creatinine (SCr) was 2.5 mg/dl(range 0.8-6.6); one pt had acute renal failure due to IgG4-TIN. 6 pts (75%) had other organ involvement by IgG4 disease. All 4 pts with available data had an increased serum IgG4 level. On biopsy, 4 pts (50%) had concurrent IgG4-TIN with increased IgG4+ plasma cells. Two pts had other glomerular disease in addition to MGN: one had diabetic glomerulosclerosis and the other had IgA nephropathy. One pt had a segmental endocapillary proliferative pattern with MGN. By immunofluorescence, glomeruli showed granular glomerular capillary loop staining for IgG, C3, kappa and lambda. One biopsy showed tubular basement membrane deposits. Electron microscopy revealed stage I-II MGN.

Follow-up data were available for all pts, at a mean of 37 mos(range 1-184). 6 of 8 pts were treated for MGN. Two pts received prednisone monotherapy, 2 prednisone followed by mycophenolate mofetil (MMF), one rituximab and MMF, and one prednisone and cyclophosphamide. The 2 untreated pts developed end-stage renal disease within 1 mo and 3 yrs; one of these pts was transplanted, without clinical evidence of recurrent disease and with stable SCr 11 years post-transplant. The mean SCr at follow-up of the other 6 pts was 1.4 mg/dl(range 0.8-2.5) and proteinuria was 1.4 g/day range 0.17-3.1). 2/6 pts showed a complete response of proteinuria to treatment and 3 showed a partial response; the other had heavy proteinuria at 1 mo follow-up. The pt with acute renal failure and TIN showed marked improvement in SCr with prednisone monotherapy.

Conclusions: MGN should be included in the spectrum of IgG4-RD and should be suspected in pts with nephrotic-range proteinuria. MGN can occur concomitantly with IgG4-TIN. Treatment with immunosuppressive drugs may improve proteinuria and may improve renal function in pts with decreased renal function.