Analytical Cellular Pathology 34 (2011) 197–203
DOI 10.3233/ACP-2011-0021
IOS Press

Poster Session

Optimization of detection of complex cancer morphology using the SIVQ pattern recognition algorithm

Jason Hipp[1], Steven Christopher Smith[1], Jerome Cheng[1], Scott Tomlins[1], James Monaco[2], Anant Madabhushi[2], Priya Kunju[1] and Ulysses J. Balis[1]

[1]*University of Michigan, Department of Pathology, Ann Arbor, MI, USA*
[2]*Rutgers The State University of New Jersey, Department of Biomedical Engineering, NJ, USA*

Background: Image analysis algorithms, coupled with maturing digital whole slide imaging (WSI) technology, holds promise to provide tools for morphometric quantitation in surgical pathology. However, implementation of such strategies will require development and optimization of pattern recognition algorithms adaptable to diseases showing complex architectural features and cytologic atypias. One such example is urothelial carcinoma (UC), of which the aggressive micropapillary variant (MPUC), an aggressive variant of UC which is frequently under-recognized causing diagnostic difficulties. Herein, we demonstrate the potential of a recently described pattern recognition algorithm and its application to this challenging use case.

Methods: We have recently reported SIVQ (Spatially Invariant Vector Quantization), an algorithm that uses ring vector predicates for pattern recognition (Hipp and Cheng, 2010). However, the relative contributions of key SIVQ ring parameters have not been fully characterized. Consequently, we systematically tested SIVQ ring parameters for detection of micropapillary nests in fields of a classic case of MPUC by comparing pattern match quality scores at pixels inside and outside of a pathologist-determined “ground truth”, using the receiver operating characteristic (ROC) curve.

Results: To standardize ring parameter optimization, we tested various ring diameters, number of subrings, and inter-ring rotational “wobble” angles. First, we modulated the number of sub-rings (skipping every other ring) from 0 to max (diameter in pixels-1), finding incrementally increased AUC performance (from 0.66 to 0.86). Secondly, increasing ring vector diameter (3–25 pixels) demonstrated initial improvement through 11 pixels, then degradation in performance, identifying an optimal ring size of 11 (max AUC 0.82). In contrast, adjusting the angle of inter-ring “wobble” from minimum to maximum (1–180 degrees) showed little effect on the AUCs (<0.01 variation in AUC).

Conclusions: Optimization of SIVQ can yield impressive performance for detection of complex tumor architectural features. Using a novel iterative discovery workflow applied to this use case of MPUC tumor nest detection, we found that maximal subrings showed better ROCs, identified an optimal ring diameter, and identified minimal contribution of inter-ring “wobble” to performance. This strategy constitutes the first description of an algorithm capable of histologic identification of MPUC and provides a model workflow broadly adaptable for future applications.

Tissue refractive index as an objective and quantitative measure of pathologic processes

Zhou Wang[1], **Rajyasree Emmadi**[2], Krishnarao Tangella[3], Andre Balla[1], Shamira Sridharan[1] and Gabriel Popescu[1]

[1]*Department of Electrical and Computer Engineering, University of Illinois Urbana-Champaign (UIUC) and The Beckman Institute for Advanced Science and Technology, IL, USA*
[2]*Department of Pathology, University of Illinois at Chicago (UIC), IL, USA*
[3]*Provena United Samaritans Medical Center, Danville, IL, USA*

Background: Traditional tissue examination and pathology diagnosis comprises light microscopic evaluation of formalin-fixed paraffin-embedded (FFPE)

2210-7177/11/$27.50 © 2011 – IOS Press and the authors. All rights reserved

sections with various chemical stains. This is, however, a fairly subjective process with inter and intra-observer variability. Quantitative phase imaging (QPI) of unstained FFPE tissue sections provides objective, label-free, highly sensitive and quantitative data based on the intrinsic tissue refractive index. We developed Spatial Light Interference Microscopy (SLIM), a new white-light QPI method that combines Zernike's phase contrast microscopy and Gabor's holography. Using SLIM we imaged entire unstained prostate tissues with a side-by-side comparison with adjacent sections of H&E stained slides.

Methods: Eleven prostate biopsies from 9 patients were imaged with both SLIM and traditional light microscopy. We utilized SLIM to analyze 4 μm sections of FFPE prostate tissues that had been de-paraffinized and placed in xylene. Three successive slices were stained with H&E and immunohistochemical stains using antibodies against Cytokeratin 34 beta E12 (high molecular weight CK903) and Alpha methylacyl-CoA-racemase (AMACR, p504s) and imaged with the same microscope (10 × objective) via the bright field channel equipped with a color camera. For each biopsy, pathologist identified regions of normal and cancer were designated the gold standard and compared to the phase shift variance data obtained by SLIM.

Results: The spatially resolved scattering map obtained by SLIM showed very good correlation with the designated benign and malignant areas. Regions of high variance (short scattering mean free path) corresponded to the darker staining associated with tumor in H&E sections. Our findings indicate that prostate cancer renders tissue more inhomogeneous, making it more strongly scattering than adjacent benign tissue. These findings were further confirmed by anisotropy factor measurements wherein malignant tissues consistently exhibited higher g values. A mode vs fluctuation contrast histogram constructed from the SLIM data separates prostate cancer from normal with 100% accuracy as tested on 100 tissue regions from 11 different biopsies.

Conclusions: Our data demonstrate that the refractive index distribution of tissue is a valuable intrinsic marker of disease and can set the basis for a new generation of computer-assisted, label-free histopathology, to enable earlier disease detection, more accurate diagnosis, and high-sensitivity screening.

Mid-Infrared spectroscopic imaging for breast tissue histopathology: Towards 'stainless staining'

M.J. Walsh[1], D. Mayerich[1], E.L. Wiley, **R. Emmadi**[2], A. Kajdacsy-Balla[2] and R. Bhargava[1]

[1]*Department of Electrical and Computer Engineering, University of Illinois Urbana-Champaign (UIUC) and The Beckman Institute for Advanced Science and Technology, IL, USA*
[2]*Department of Pathology, University of Illinois at Chicago (UIC), IL, USA*

Background: Histopathology is the gold standard for disease diagnosis. Current histopathological techniques use a panel of special stains and immunohistochemistry (IHC) to assess tissue architecture, determine cell types present and to classify cancers. Mid-Infrared (IR) spectroscopic imaging is a novel approach to derive chemical images from tissues based on their inherent biochemistry.

Methods: Mid-IR images were obtained from over 200 individual patients using breast tissue microarrays. Serial sections were stained with a panel of 13 routinely used special stains and IHC stains. A modified Bayesian classifier was built to assign image pixels to the correct cell types and Artificial Neural Networks (ANN) to replicate staining. Using Mid-IR imaging coupled with the modified Bayesian classifier it was possible to segment breast tissue into the main 8-cell types of breast tissue from a single unstained tissue section.

Results: The sensitivity and specificity as measured by average Area Under the Curve (AUC) were very high (AUC = 0.9). Mid-IR imaging coupled with ANN demonstrated that it was possible to accurately reproduce the staining of the panel of stains, all in a single unstained slide.

Conclusions: Mid-IR imaging coupled with Bayesian classification and ANN could potentially be a very valuable tool as an adjunct to current histopathological procedures, with the ability to take a single unstained tissue section and give a decision on the cell types present and also to replicate staining patterns. This approach could be particularly advantageous where limited histological and cytological specimen is available for analysis. Moreover, it is amenable to quantitative analysis of each component. This novel approach promises to revolutionize and expand the role of the pathologists in both research and tissue diagnosis.

Medical School Pathology education supplemented with web-based virtual microscopy

Rajyasree Emmadi, Amy Y. Lin, and Andy V. Pham

Department of Pathology, University of Illinois at Chicago (UIC), IL, USA

Background: The year 2 Medical student Pathology practicum at UIC, designated Small Group Discussion (SGD), comprises 184 students in groups of 14–16 each. Traditionally the practicum has involved students examining and learning morphologic pathology by viewing glass slides through the microscope, with accompanying instruction, in 2-hour sessions. We decided to introduce web-based virtual microscopy to the SGDs to augment teaching and facilitate self-paced student learning.

Methods: Selected glass slides were scanned using the Aperio ScanScope (Aperio, Vista, CA), to obtain the virtual slide images and interactive cases were created using Digital Slide Box (DSB) software (SlidePath, Dublin, Ireland). Each virtual slide was annotated with key microscopic features of the case. Each case, in turn, was accompanied by a narrative, which included a clinical history, physical examination findings, and gross and microscopic descriptions. A total of 39 virtual slide sets were utilized for the current academic year. Hyperlinks within the narrative are available to integrate clinical photographs, gross photographs, imaging studies, multimedia, additional virtual slides or slide annotations to the case. At the end of each module, self-assessment quizzes helped students test their understanding and identify individual weaknesses.

Results: These interactive, web-based virtual microscopy cases were provided to the M2 students the week prior to the actual SGD. This allowed the students to study the material at leisure and without a time constraint. At the actual SDG, the students were able to concurrently view different glass slides of the specific disease process as well as re-review the online material. While initially the in-class bandwidth slowed the online review noticeably, this was resolved by effecting a change in the DSB server configuration. An informal feedback showed that the students were very receptive to this new technology and found these self-study cases useful in increasing their recognition and understanding of pathologic processes in diseases. They particularly liked the 'anytime, anywhere' access and the ability to dispense with the microscope.

Conclusions: An interactive, web-based virtual microscopy case study set makes Pathology more accessible and inviting to Medical students. In the next academic year we plan to expand the virtual microscopy content.

Toward an annotated digital Multiphoton Microscopy (MPM) histology atlas of fresh human bladder biopsies for intra-cystoscopy guidance in bladder cancer diagnosis

Sushmita Mukherjee[1], Manu Jain[2], Brian D. Robinson[2,3], Joshua Sterling[1], Douglas S. Scherr[2], Bekheit Salmoon[1], Frederick R. Maxfield[1], Warren R. Zipfel[4] and Watt W. Webb[5]

[1]*Department of Biochemistry, Weill Cornell Medical College, New York, NY, USA*
[2]*Department of Urology, Weill Cornell Medical College, New York, NY, USA*
[3]*Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY, USA*
[4]*Department of Biomedical Engineering, Cornell University, Ithaca, NY, USA*
[5]*School of Applied and Engineering Physics, Cornell University, Ithaca, NY, USA*

Background: Hematoxylin and eosin (H&E)-stained sections obtained from formalin-fixed specimens is the current gold standard for histopathological diagnoses. While these methods are highly reliable, they have lengthy time requirements (processing, sectioning, staining, and reading by pathologists). Although efforts at digitization of glass slides are in progress, most pathologists still read analog slides; therefore, automated morphometry and real time online consultations are rare. Furthermore, the 2-dimensional nature of histology slides precludes assessment of 3-dimensional tissue architecture without time consuming serial sectioning.

Methods: Multiphoton microscopy (MPM), a nonlinear imaging technology, generates 3-dimensional histology of the tissue at sub-cellular resolution and at depths up to 0.5 mm below the tissue surface. This allows nearly instant imaging of fresh (unfixed, unsectioned, and unstained) tissue based on spectrally resolved intrinsic tissue emission (ITE) signals: (1) autofluorescence from cell cytoplasm components and

elastin fibers; and (2) Second Harmonic Generation (SHG), a nonlinear scattering signal from collagen bundles and oriented microtubules. Using a single excitation wavelength and collecting emission signals using wavelength bandpass filters, SHG and various autofluorescence components can be separately acquired, analyzed and color-coded for easy visualization.

Results: We analyzed *ex vivo* tissues from human bladder biopsies by MPM and compared our diagnostic impressions with gold standard hematoxylin and eosin stained sections of the same specimens. MPM images alone provided sufficient details to classify most lesions as either benign or neoplastic, using the same basic diagnostic criteria as histopathology, namely architecture (flat or papillary) and cytologic grade (benign/low grade or high grade). We have begun the generation of a pathologist-validated and annotated digital atlas containing both the MPM image sets and the corresponding H&E histopathology.

Conclusion: A validated digital MPM atlas may provide intra-cystoscopic guidance to urologists *in situ*.

Spectral sensing method for practical use

Yasuhiro Fukunaga, Saori Shimizu, Kensuke Ishii, and Kosei Tamiya

Olympus, Inc., Japan

A pathology imagery interpretability rating scale for virtual microscopy

Peter Kragel and Bill Oliver

Department of Pathology and Laboratory Medicine, East Carolina University, Greenville, NC, USA

Automated 3D-reconstruction of histological sections

Maristela L. Onozato, Mark Merren, and Yukako Yagi

Department of Pathology, Massachusetts General Hospital, Boston, MA, USA

Introduction: Three-dimensional (3D)-reconstruction from serial sections is a valuable tool to provide structural and morphometric data of complex structures however, unlike virtual sections such as from radiology scanned images, 3D-reconstruction from physical histological sections are more challenging due to the distortion that occurs from processing and positioning each section on each individual slide. Objective: In this work we tested whether a completely automated tissue processing system with automated sectioning machine and slide scanning system could generate precise 3D-reconstructions of tissues that could match the images obtained with an optical tomographic device.

Methods and results: Human lung and heart and rat kidneys were scanned with large-field-optical coherence tomography (LF-OCT, LLtech Inc., Princeton NJ) in order to obtain micrometer resolution images of unprocessed biopsy tissues. The tissues were then embedded in paraffin and sectioned with Kurabo-Automated tissue sectioning machine. Serial sections were then automatically stained and scanned with a Whole Slide Imaging device. Images were 3D-reconstructed with inbuilt software and compared with the unprocessed images obtained with LF-OCT. 3D-reconstructed images of human lung and heart showed a very close structural details to the images obtained with LF-OCT. 3D-reconstructed rat kidney revealed details of spatial distribution and structural interaction of the nephron different segments including the vascular pole of the glomerulus however some minimal distortion could not be prevented by this all-automated system.

Conclusion: Technology advances are allowing simpler ways of obtaining 3D images from 2D slides therefore a better interpretation and analysis of complex structures such as the nephron with details that can not be provided by light optical imaging systems however still some congruency details and imaging corrections have to be implemented.

Automated sectioning machine for paraffin blocks

Maristela L. Onozato[1], Stephen Hammond[2], Mark Merren[1] and Yukako Yagi[1]

[1]*Pathology Information and Communication Technology (PICT) Laboratory, Department of Pathology, Massachusetts General Hospital, Boston, MA, USA*

[2]*Department of Pathology, Boston University, Boston, MA, USA*

Background and significance: Microtomy has been a limiting factor for the development of a fully automated system for tissue histology. In this work we present a novel robotic paraffin-sectioning machine and we compare the automated sectioned slides with traditional manual sections.

Material and methods: A total of 46 blocks were manually or automated sectioned at 4 µm and then hematoxylin-eosin (H&E)-stained. Sections were scored by two blinded-professionals based on the presence of technical imperfections or irregularities that could interfere with pathology evaluation. The score ranged from1 to 4, with scores closer to 0 reflecting perfect sections. Immunohistochemistry was also performed in breast tissue to confirm that antigenicity was not affected by the procedure.

Results: Automated sectioned slides showed remarkable quality compared with manual sections with fewer imperfections (automated score 0.87 ± 0.07 vs. manual 1.49 ± 0.07, $p < 0.001$) however manual sectioning for the 46 blocks was almost two-fold faster than the robotic system. The robotic-system showed the best performance for serial sections with slides showing stable thickness and same orientation allowing easy stacking and 3D reconstruction. Breast tissue showed preserved antigenicity demonstrated by immunostaining for human epidermal growth factor receptor-2 (HER-2), estrogen receptor (ER) and progesterone receptor (PgR).

Conclusion: Automated robotic microtome can perform high quality sections of tissues of different consistencies without compromising their antigenicity. The turnaround time is the limiting factor that needs to be improved for its implementation in a histology laboratory.

Balancing image quality and compression factor on special stains in Whole Slide Images

Anurag Sharma[1], Pinky Bautista[2] and Yukako Yagi[2]

[1]*Department of Pathology, NEC Laboratories, Princeton, NJ, USA*
[2]*Department of Pathology, Massachusetts General Hospital, Boston, MA, USA*

Background: The image compression in is measured in quality factor (QF). Higher QF (100 being the best) provides better quality but adversely affects the resources. Most scanners have provision to set compression quality factor (QF) for the images. It is becoming more important to use the technologies to balance the quality with overheads especially in high volume scanning where the daily average is over 100 slides for a variety of purposes. To find a QF value that is a perfect fit for our daily high volume scanning use and provides a practical balance between quality and performance was seen as 80 for H&E, 50 for Reticulin and 30 for quite a few special stains. Now we extend the earlier experiments to investigate the effect of QF by computing the difference in color.

Technology: Three unique systems scanned 16 whole slides with six QFs in 12 stains: Trichrome, PAS, Retic, GMS, Geimsa, BrownHopps-Gram, Steiner, Worthin-Starry, Mucicarmine, Elastic, PAS-D, and Congo-Red.

Design: This experiment consisted of two sets of eight special stains slides each. The slides containing human tissues and mouse embryo were scanned with 0.33~0.50 um/pixel resolution in three scanners at seven QF levels: 30, 50, 60, 70, 80, 90 and 100. This experiment generated over 200 images. Since many special stains deal more with color difference, we tried to benchmark the same. The LAB color values were calculated on specific points within regions of interest and also for the background. The difference between these values was computed to understand the pattern for different stains. The process was repeated for various quality factors.

Results: In earlier experiments with H&E and special stains with human observation including that of a pathologist, we found that the compression artifacts were more visible at lower QFs. However this time we focused to see if the image quality was good enough to show the color difference between region of interest and the background. Though the difference was dependent on the stains it was still above the distinguishable value for average human eye. Most special stains images were still acceptable at QF 30 except for the stain Reticulin where the lowest acceptable QF was 50.

Multispectral enhancement towards digital staining

Pinky A. Bautista and Yukako Yagi

Pathology Information and Communication Technology (PICT) Laboratory, Department of Pathology, Massachusetts General Hospital, Boston, MA, USA

Background: Digital staining can be considered as a form of image enhancement whereby the image pixels are colored to simulate the effect of chemical stains. That is, unlike the general forms of image enhancement paradigms, both the image backgrounds and objects of interest should be colored appropriately to reflect the reactions of the objects when subjected to physical staining. We introduced a digital staining method by introducing a modification to a multispectral enhancement method previously proposed.

Method: In the previous multispectral enhancement method a shifting factor is tntroduced to the original spectrum of the pixels. This shifting factor is a product between the spectral residual-error and the difference between the spectrum of the target spectral- color and the average spectrum of the pixels in the image. To implement digital staining we introduced a spectral transformation process prior to spectral shifting. Moreover, the shifting factor is also modified. Instead of considering the average spectrum of the image pixels, the transformed spectrum of each pixel was considered.

Results: The digital staining method was applied to the multispectral images of liver tissue stained with hematoxylin and eosin (H&E) dyes. The H&E stained images were digitally converted to their Masson's trichrome stained counterparts- digital staining. The enhanced H&E stained images show correlation with their Masson's trichrome stained images counterparts, which were physically stained, i.e., the collagen fiber areas were colored blue.

Conclusions: Experiments on the digital transformation of hematoxyllin and eosin (H&E) stained images to their Masson's trichrome stained equivalent show that the present digital staining approach is feasible. Further improvement on the method is expected to make it more robust to spectral variations.

A method for segmentation and quantitative assessment of lymphatic vessels in histological images of serous ovarian carcinomas

M. Frydman[1], V. Kovalev[2], A. Konchic[2], S. Kupryian[1]

[1]*Minsk City Hospital for Oncology, Minsk, Belarus*
[2]*United Institute of Informatics Problems, Belarus National Academy of Sciences, Minsk, Belarus*

Background: For many serous ovarian carcinomas, the invention of lymphatic endothelial markers has enabled the unambiguous characterization of lymphangiogenesis during the tumor progression.

Aims: To present an original technique of computerized segmentation of lymphatic vessels for their easy and unbiased count.

Materials and methods: The method was implemented in form of original software package that calculates quantitative descriptors of lymphatic vessels. The input data are expected to be original histological images processed with the D2–40 endothelial marker while the results are the set of quantitative features of lymphangiogenesis. The method of computerized segmentation of lymphatic vessels presented in this study is aimed at the quantitative assessment of lymphatic network. It is based on the analysis of color space of histological images followed by image binarization and calculation of quantitative features characterizing the lymphatic network.

The image segmentation procedure consists of the following major steps.

- Conversion of the original RGB color space to the HSL color representation (HSL stands for the hue, saturation, and lightness respectivelly).
- Highlighting image pixels that represent vessels. A pixel in this case is a part of a lymphatic vessel if its color components satisfy the following condition: $H < 0.12$ and $L < 0.7$.
- Removing noise image components that include objects with an area of less than 7 pixels.

The analysis of binary image resulted from the above steps consists of the counting features which describe the relative image area occupied by vessels, the uniformity of the vessel's distribution and the relative proportion of large vessels.

Results: The computerized procedure of histological image analysis resulted in computing the following quantitative features.

(a) The relative area occupied by the vessels. It is computed as the total amount of vessel pixels divided by the total number of pixels in the image.

(b) The homogeneity of the distribution of vessels network over the image (tissue sample) space. For computing this feature, the whole image is subdivided into 100×100 identical fragments. For each fragment, the degree of distribution uniformity is calculated. In our case, the entropy is determined in the following way: $H = -\Sigma Pi * \ln(Pi)$, where Pi is the frequency (probability) of hitting a pixel in the i-th fragment. This value varies from zero (all pixels are situated in one single fragment) up to

one (homogeneous pixel distribution over all the fragments).

(c) The relative area of small vessel. For computing this value, the pixels belonging to the image boundary are removed by a standard erosion morphological operation. Then the ratio of the removed area to the original vessel area is calculated. This feature ranges from zero (all the vessels are small and separated from each other) to one (the image does not contain any small vessels and/or noise appearing like vessels).

(d) The relative proportion of large vessels. It is computed similar to the previous feature except that the vessels area remaining after filtration repeated six times is divided to the original vessel area.

The above procedure was thoroughly tested on a trial set containing 5000 images of histological samples of about 100 patients stained with the help of D2–40 endothelial marker.

Conclusion: Future studies are necessary to determine whether intratumoral lymphatics are restricted only to certain cancer types and whether their presence in tumors has prognostic significance.