Presenter's Name: Coats, Jennifer

Additional Author(s): Dammak S, Lin SXJ, Ward A, Cecchini MJ

Abstract Title: Analysis of tumour cellularity variance in simulated core needle biopsy specimens across resected lung cancer cases.

Abstract:

Introduction: Core needle biopsies (CNB) are routinely used for the diagnosis of lung cancer. Imaging modalities, such as magnetic resonance imaging (MRI), ultrasound, and computed tomography (CT) scans, are used to guide the CNB into the target lesion. Currently, CNB are frequently targeted at the center of the tumour unless there is a necrotic core. We hypothesize that the center of the tumour is not always the most cellular given the observed variance in tumours seen in clinical practice. With increasing numbers of molecular tests performed on these small biopsies, it is critical that the tumour cellularity is optimized to ensure there is sufficient material for molecular testing.

Methods: Digital slides were randomly selected from the TCGA LUAD (lung adenocarcinoma) n=100 and LUSC (lung squamous cell carcinoma) n=50 datasets. The area of tumour was annotated and a total cell detection was performed in QuPath. An object classifier was then introduced based on annotations (reviewed by a thoracic pathologist) to distinguish tumour cells from surrounding inflammatory and stromal cells. We then simulated ideal core needle biopsies (measuring 0.25 mm x 2.5 mm) and tiled these across the entire slide. The number of tumour cells, total cells and tumour cellularity was recorded within each simulated core. The percent tumour cellularity and the number of tumour cells was then mapped back across the tumour and visualized with density maps.

Results: In the squamous cell carcinoma cases, the percent tumour cellularity was highest in the central region in 25% of the cases, the intermediate region in 48% of the cases, and the peripheral region in 27% of the cases. Similarly, in the adenocarcinoma cases, the percent tumour cellularity was highest in the central region in 19% of the cases, the intermediate region in 51% of the cases, and the peripheral region in 30% of the cases.

Discussion: The majority of the time the most cellular cases were in the intermediate area between the center and the periphery of the tumour. However, there was a spectrum of findings with some cases having the highest cellularity at the periphery and lower cellularity in other areas. This highlights the need for future studies to correlate these findings with imaging data to develop more sophisticated algorithms to better guide the targeting of core needle biopsy sampling.

POSTER PRESENTATIONS 3
3B: DIGITAL PATHOLOGY

Presenter's Name: Dammak, Salma

Additional Author(s): Cecchini MJ, Ward AD

Abstract Title: Predicting Tumor Mutational Burden from H&E Slides of Lung Squamous Cell Carcinoma

Abstract:

Introduction: The PD-L1 score is used to guide treatment decisions, but it does not accurately predict response to immunotherapy in all cases. While adding tumor mutational burden (TMB) to PD-L1 improves response prediction, it is costly and typically requires high tumor cellularity. Previous studies have demonstrated that genomic information such as driver mutations, are encoded in the morphologic appearance of cancer cells1. However, there are not currently any established means to visually distinguish them. In this study, we hypothesized that a neural network could be trained to distinguish the two based on digitized standard-of-care H&E slides.

Methods: We utilized digital slides of tumour resections from the Cancer Genome Atlas lung squamous cell carcinoma. The dataset had 50 patients across 30 centers which we split into 30 training and 20 testing slides, each from a unique set of centers. We calculated the TMB with a 10 mutations/Mb threshold utilized to separate TMB-High and Low cases. We explored various different model parameters using the training set only, then fixed the model and tested it on the test set.

Results: The selected model is VGG162, trained using the technique of transfer learning, and had an area under the receiver operating characteristic curve of 0.65, accuracy of 65%, sensitivity of 77% and specificity of 43%.

Discussion: This study suggests that complex genetic features of a tumor are encoded in the morphologic appearance on H&E slides, and this is the first study that shows that TMB specifically can be detected in the morphology of squamous cell carcinoma on H&E slides across multiple centers. Further, it shows that even with a small training set, it is possible for a neural-network-based model to detect this relationship. This motivates additional work in this direction to build a system that can be used in the future to help physicians decide which patients with squamous lung carcinoma would benefit from immunotherapy.

1. Nat. Med., 2018 Oct;24(10):1559-1567
2. IEEE Trans. Pattern Anal. Mach. Intell., 2016 Oct;38(10):1943-1955

Presenter's Name: Keow, Samantha

Additional Author(s): Lin SXJ, Cecchini MJ

Abstract Title: How tall are you? Using digital pathology to automatically quantify and characterize the tall cell variant of papillary thyroid carcinoma.

Abstract:

Introduction: Tall cell variant (TCV) papillary thyroid carcinoma is a subtype of thyroid cancer with an aggressive biologic behaviour and poor prognosis when compared to classical papillary thyroid carcinoma (PTC). The 2017 WHO classification of tumours of endocrine organs defines TCV as a papillary carcinoma where at least 30% of tumour cells have a length-to-width ratio of 2-3 to 1. Visual estimation of tumour cells remains the gold standard for diagnosis. However, use of image analysis and machine learning to determine length-to-width ratios provides a novel tool for absolute quantification of tumour cells with tall cell criteria. Here we compare the accuracy of automated cell detection with manual cell measurement in cases of papillary thyroid carcinoma.

Methods: Cases of papillary carcinoma (n=20) were randomly selected from the Cancer Genome Atlas (TCGA) PTC database, including TCV (n=10) and non-TCV (n=10). All cases were reviewed by a Head and Neck pathologist (MJC). Cell detection was performed using QuPath with parameters optimizing cell expansion and nuclear fragmentation. Representative tumour and non-tumour areas were used as training data for an object classifier. Calliper values were taken from detected cell parameters to estimate and stratify cell measurements by length-to-width ratio. Calliper-generated ratios were then compared to manual cell measurements to determine the efficacy of the automated approach. Results: The object classifier accurately identified 80.2% of cells that had a length-to-width ratio of 2:1 or greater within a 5% margin of error. There was a 14.7% difference between the mean ratios of the manually classified cells and the automatically detected cells. When the automated system was applied to 10 TCV and 10 non-TCV PTC cases, there was a trend towards an increased number of tall cells calculated in cases of TCV PTC.

Discussion: We were able to demonstrate that TCV could be distinguished from non-TCV with moderate accuracy using automated cell detection software. However, the object classifier was only tested against a single representative slide in each case of PTC. Future work remains to be completed with validation against all representative tissue in each case, and further refinement of cell border detection before we can validate the established WHO-defined diagnostic criteria.

POSTER PRESENTATIONS 3
3B: DIGITAL PATHOLOGY

Presenter's Name: Lam, Victor

Additional Author(s): Misra T, Wu NJ, Erem AS, Cecchini MJ

Abstract Title: Digital Quantification of Tumor Density in Relation to the Grading System in Head and Neck Squamous Cell Carcinoma

Abstract:

Introduction: Worldwide, head and neck cancer accounts for 900,000 cases annually, with head and neck squamous cell carcinoma (HNSC) as the most prevalent form. Currently, staging is based on size and spread of the tumor which may be a surrogate measure for the cellularity of the tumor. Cell counting to stage tumors is unfeasible for pathologists, however, our previous work demonstrated the feasibility of image analysis tools to facilitate quantification of cells in digital histology slides. Although separate systems of staging and grading correlate with patient outcome, the relationship between these prognostic methods is unclear in HNSC. In this study, we hypothesize that high tumor densities correlate to high-grade histologies.

Methods: 21 digital slides were obtained from The Cancer Genome Atlas (TCGA) HNSC dataset. Areas of tumor were annotated using QuPath and reviewed by an anatomical pathologist. Annotations were then used to train machine learning-based cell detection algorithms to obtain total tumor cell counts. Accounting for the surface area, tumor density (cells/mm2) was used to normalize the data. Each digital slide was divided into 1260 µm x 1260 µm grids and treated as individual datum. Three grids of relative average tumor counts from each case were used for a total of n=63.

Results: Automated tumor classification was accurate as reviewed by an anatomical pathologist. In this preliminary work, significant correlation was observed between tumor density and grade using Kruskal-Wallis non-parametric test (p=0.036). Dunn's post-hoc comparison revealed significant differences between the well and the poorly differentiated groups (p=0.005). Significant difference was also observed between moderate and poor histological grading (p=0.023). Additionally, there was a variability of tumor cellularity ranging from 1674 to 14031 cells in the 21 cases. This variability was also noted in the tumor cell density within the tumor due to heterogeneity and tumor necrosis.

Discussions: Digital tools facilitate the automation of cell classification for tumor density efficiently. In this preliminary analysis we observed a significant trend towards higher density associated with high grade tumors. Ongoing/future work will explore this relationship in greater detail. Given the availability of genomic and gene expression data from these TCGA datasets, the molecular underpinnings of tumor cellularity can also be explored.

Presenter's Name: Lin, Sherman

Additional Author(s): Samsoondar JP, Keow S, Pokharel BB, Tan D, Martinez-Acevedo J, Pham M, Wu NJ, Misra T, Lam VHK, Sansano I, Cecchini MJ

Abstract Title: Digital Quantification of Tumor Cellularity as a Novel Prognostic Feature in Lung Adenocarcinoma

Abstract:

Introduction: Lung cancer is staged based on the size of the tumor and involvement of other structures. This staging may be a surrogate measure for the number of cells present in the tumor. The recently updated grading system for lung adenocarcinoma assesses the presence of high risk architectural patterns, which tend to have more complex cellular growth. Counting individual tumor cells is impractical for a pathologist using a conventional light microscope. Image analysis tools applied to digital slides can be utilized to automate the quantification of lung adenocarcinoma. We hypothesize that tumor cellularity can be used as a novel prognostic tool in lung cancer that integrates quantification of high risk architectural patterns.

Methods: Digital slides (n=102) from the Cancer Genome Atlas (TCGA) lung adenocarcinoma (LUAD) dataset were obtained and analyzed in QuPath. Representative areas of tumor were annotated and reviewed by a thoracic pathologist, the annotations were used as training data for a random trees based object classifier that utilized detected cell features to identify and quantify tumor cells across entire slides. This was normalized with the surface area of the tumor present on the slide to provide a measure of tumor density. The overall total cellularity was calculated by combining the size of the grossly measured tumor with the tumor density. Major histologic patterns in representative panels were determined by a thoracic pathologist and were compared with the tumor density of the tile. The overall and progression free survival was compared between groups of high and low tumor cellularity.
Results: High-grade histologic patterns had a significantly greater tumor density compared with other patterns of lung adenocarcinoma. A trend between survival and cellularity was identified and a cut-off of 5.5 x 1010 cells was found to predict outcome. Cases with a low cellularity had an improved progression free survival (HR 0.21; 95% CI 0.096-0.47) and overall survival (HR 0.25; 95% CI 0.088-0.7) compared with cases that had higher cellularity.

Discussion: Tumor cellularity represents a novel prognostic tool in lung cancer that takes into account both the size and composition of the tumor. Use of advanced image analysis tools allows for the automation of this task in a simplified and efficient manner. Future work will seek to validate these findings in additional larger datasets to refine the classification of tumors by cellularity.

POSTER PRESENTATIONS 3
3B: DIGITAL PATHOLOGY

Presenter's Name: Pearce, Joanna

Additional Author(s): Biswas S, Pasman E, Chakrabarti S, Marshall H, Cecchini MJ

Abstract Title: Development of a Novel Ultrasound Device to Detect Lymph Nodes in Colorectal Cancer Pathology Specimens

Abstract:

Introduction: Colorectal cancer (CRC) is one of the most diagnosed cancers in Canada, and incidence rates have had a global rise since the 1990s. Pathology labwork has seen a correlated jump, with an estimated 21-23% increase in workload. Already, CRC gross examination is considered more labour-intensive and time-consuming than other specimens. For proper Tumour-Node-Metastasis (TNM) classification, it is vital to identify all lymph nodes (LNs) in a specimen, which directly affect cancer treatment and prognosis. Identification of LNs during gross assessment is crucial but challenging, as LNs can be small and difficult to find via manual palpation and dissection. In this project, we have developed a device that utilizes ultrasound to detect LNs in resected tissues.

Methods: Animal mesenteric tissues are being used for validation of this device. The initial prototype involves a robotic gantry, equipped with ultrasound imaging, that has the potential to assist pathology staff in finding LNs in resected specimens. Validation of the ultrasound results includes collaboration with a radiologist, pathologists' assistant (PA), and pathologist. Briefly, once a scan is complete, LNs are dissected by the PA and placed on a grid, where LNs are confirmed by a pathologist. The recorded ultrasound images are reviewed by a radiologist who identifies and labels LNs present in the footage. The location of each identified LN is correlated to the coordinates in XY space where the images were captured, and this is compared to the LNs identified by the PA.

Results: Our preliminary results show the ability of the device to distinctively highlight tissue regions of interest containing pig LNs in mesenteric adipose tissue. Over 3 independent experiments, the device (with a radiologist's ultrasound review) was able to identify LN-positive regions in pig mesenteric tissues with 100% sensitivity, 90% specificity, 92% accuracy, 74% positive predictive value and 100% negative predictive value.

Discussion: The application of ultrasound imaging for detection of LNs appears promising for surgical pathology specimens and warrants further exploration into the development of a device that can autonomously search for LNs and mark their locations for extraction by pathology staff. An automated device could fundamentally improve how we identify LNs in pathology specimens, which could offer significant cost savings for hospitals and reduce the potential for repetitive strain injuries with PAs.

Presenter's Name: Pierce, Kevin

Additional Author(s): Lin SXJ, Ghafoori E, Coats J, Keow S, Lam VHK, Martinez-Acevedo J, Misra T, Pham M, Pokharel BB, Samsoondar JP, Tan D, Wu N, Cecchini MJ

Abstract Title: Pathology Annotated Tile-based High-throughput Classification Application (PATHCA) for Lung Cancer Classification

Abstract:

Introduction: In pathology and other fields, one of the greatest barriers to developing machine learning models is the need for large numbers of labelled examples for training. Traditionally, generating a collection of tumour annotations on H&E slides requires highly skilled pathologists and is an extremely time-consuming task. We have built an online collaboration platform designed to collect labelled tilesets to support the training of a supervised machine learning model. Using this approach, the task of labelling slides can be reduced to a simple image recognition task, requiring minimal supervision to generate labelled training data.

Methods: 11 undergraduate, 1 graduate student and 1 medical student without formal pathology training labelled tiles in the application. 320 tiles of dimension 570x570 μm2 were fragmented from contours of adenocarcinoma cases from the TCGA diagnostic lung adenocarcinoma dataset. Fragmented tiles were isolated in identical batches of 16 and displayed in a 4x4 grid within the application; participants were instructed to identify tiles that were positive for tumour. Each user's selection or non-selection was used to generate a group consensus on whether or not the tile contained tumour. Each user's accuracy was then evaluated with respect to the consensus. The same 320 tiles were given to a thoracic pathologist, and both the consensus and each individual were scored against the pathologist's selections.

Results: Tiles with high agreement showed the presence or absence of tumour cell nests with varying degrees of eosinophilic cytoplasm and nuclear pleomorphism whereas tiles with split consensus potentiate scenarios of normal goblet cells, single isolated tumour cells, or presence of artefacts, all showing tumour-like characteristics. The cases with high consensus showed good accuracy (84.4%) with respect to the pathologist. Cases of discrepancy between the consensus typically involved focal involvement of the tile by tumour. This is a highly efficient means for labelling with participants able to quickly identify tumour (range: 1.7 - 32.3; average 9.1 seconds per tile).

Discussion: We demonstrate that a clinically-oriented collaborative tool can simplify the identification of tumours into an image recognition task performed by non-pathologist observers to generate labelled training data. We anticipate this application can remove barriers in generating labelled tilesets for machine learning models in pathology.

POSTER PRESENTATIONS 3
3B: DIGITAL PATHOLOGY

Presenter's Name: Skeba, Danielle

Additional Author(s): Asfaha S, Shin AE, Cecchini MJ

Abstract Title: Digital Characterization of Bowel Damage in Chemical- and Bacterial-Induced Experimental Colitis in Mice

Abstract:

Crohn's disease and ulcerative colitis comprise inflammatory bowel disease (IBD), an idiopathic disease with genetic and environmental influences. IBD is becoming increasingly prevalent in the global population. To facilitate mechanistic studies of IBD, the colitis phenotype is often induced in mice using orally administered dextran sodium sulfate (DSS). Other models of colitis are achieved with the use of oxazolone, TNBS, Citrobacter rodentium and doxorubicin. Currently, researchers working with histologic colitis samples from these models manually identify distinguishable features of each model and quantify areas of disease activity. Manual analysis introduces inter- and intra-observer variability, as well as considerable time invested. More powerful, reliable, and refined analytical capability may be achieved using digital pathology tools. This study utilizes QuPath, an open-source whole-slide image analysis program, in conjunction with CytoMAP, a built-in MatLab tool for tissue spatial analysis, to compare and characterize the different models of colitis in mice. This method, leveraging both the cell detection and classification features of QuPath, as well as the streamlined clustering analysis pipeline of CytoMap, will extract features from each of the models of colitis. The findings of this study may contribute to the creation of a more efficient and reliable method for identifying colitis in mice. In addition, this study will contribute to research into the emerging field of digital pathology as a tool in research and potentially the clinical assessment of IBD.

Presenter's Name: Woo, Elissa

Additional Author(s): Cecchini MJ

Abstract Title: Shades of Hematoxylin and Eosin: Digital Image Analysis of Stain Variability

Abstract:

Introduction: Hematoxylin and eosin staining has been the standard for pathology laboratories around the world for over a century. Despite its long history, staining variability persists not only between laboratories but within a single lab. In some cases, subtle differences cannot be detected by standard analog quality metrics. These subtle variations create inconsistent results which present a bigger issue with the implementation of artificial intelligence amongst other new technologies. Through quantification and visualization of variation, an improved understanding of the amount of variation that is occurring along with which tissue or cell types are most greatly affected can be achieved.

Methods: Tissue control blocks containing hepatic, renal cortex, small and large intestinal tissue sections were serial sections and stained utilizing a routine hematoxylin and eosin staining procedure. A total of 40 slides were included in this study, consecutively scanned on the Aperio AT. QuPath was utilized for digital analysis by colour deconvolution and optical density. Prism graphing software was utilized for statistical analyses and visualization of the data obtained.

Results: Data represented graphically allowed for dramatic coefficients of variation to be better understood when contrasted with variation occurring in other tissue types. Data obtained for the optical density of tissue sections followed expected patterns with hematoxylin having higher mean density compared to eosin staining. All nonnuclear deconvoluted hematoxylin results were excluded. Deconvoluted eosin staining results showed to be consistently problematic across all cell types analyzed.

Discussion: Images of minimum and maximum optical density sections were included for side-by-side comparison and visualization of the span of variation that is occurring. Through analysis of both deconvoluted hematoxylin and eosin, overstaining with eosin as observed in the maximum optical density sections proved to be extremely problematic for interpretation. Further investigation of the staining procedures and the effects of the reagent change schedule would help eliminate occurrences of overstaining and over differentiation.

POSTER PRESENTATIONS 3
3B: DIGITAL PATHOLOGY

Presenter's Name: Yang, Joe

Additional Author(s): Chen L, Ling C, Zhang Q

Abstract Title: An Artificial Intelligence Based Classifier for the Diagnosis of Pseudo- and True Invasion in Colorectal Polyps

Abstract:

Introduction: As opposed to submucosal invasion (true invasion), a problematic and well-described mimic occurs when glands become misplaced in the submucosa, termed pseudoinvasion. It has posed a challenge in colorectal cancer diagnosis that sometimes cannot be overcome even by a panel of expert pathologists. In this joint work, we are investigating a two-stage deep learning pipeline using convolutional neural networks (CNN) that can automatically determine whether a patient has invasive colorectal cancer through the patient's whole-slide images (WSI) of colon polyps.

Methods: Slides from 130 cases of colon polyps including 70 cases of pseudoinvasion and 60 cases of true invasion are scanned into WSI, and annotated into 12 specific tissue categories. Then, we implement a two-stage patch-based algorithm. In the first stage, we tile the WSI into a collection of non-overlapping patches. We establish a CNN model that recognizes and classifies each image patch into 9 tissue type categories: adipose, background, debris, lymphocytes, mucus, smooth muscle, normal colon mucosa, cancer-associated stroma, and colorectal adenocarcinoma epithelium. We train our CNN model using 80% of the public NCT-CRC-HE-100K dataset and use the rest for validation. In the second stage, we use two models, a linear model and a 3-layer CNN, to aggregate the per-patch classification results into a final classification. We validate the linear model using the leave-one-out method and the 3-layer CNN using 6-Folds cross-validation on a collection of 64 WSIs.

Results: The stage-one CNN has achieved an overall accuracy of 98% on the validation set which consists of 20,000 image patches from H&E stained WSIs. In stage two, the linear classifier has successfully differentiated 83% of the WSIs into the true invasion and pseudo invasion. The 3-layer CNN has achieved an average accuracy of 88% in invasion classification during 6-Folds cross-validation.

Discussions: Although our model has fulfilled the initial goal, often the second stage classifier produces a correct final prediction based on poorly classified image patches from the first stage, which sheds doubt on the reliability of our model. We believe that the public dataset is insufficient in predicting pseudo and true invasion. Future works will be done to refine and re-label image patches for the task at hand, and to improve the speed, reliability, and accuracy of our overall predictive model.

A live demo of our model will be shown.