2020 has been a challenging but rewarding year for the Department of Pathology’s Digital Imaging Laboratory. Located at the Josie Robertson Surgical Center since its inception in 2017, the laboratory has served as an incubator to explore, develop and evaluate new technology to advance medical imaging in a clinical setting and actively engage vendors to improve the technology and develop clinical applicability. Through collaborations with research and clinical departments (e.g., Surgery, Radiology, Medical Physics, and Information Systems (IS) groups), our lab enhances the assessment and creates opportunities for multidisciplinary applications.

Our current research efforts have one central goal: to integrate digital imaging and computational pathology data with other specimen-related data (genomics, proteomics, radiographic imaging, etc.) to further enrich our knowledge of disease. This will bring an unprecedented breadth and depth of information to each individual case and yield a comprehensive, multidimensional analysis that would otherwise be impossible.

The Pathology Digital Imaging team displayed an overwhelming amount of dedication throughout the pandemic and their hard work resulted in substantial progress in each of our projects, multiple publications in peer-reviewed journals and invitations to present at international conferences. Additionally, our team has expanded in size and is now also located in the recently opened Joy Building at 61st Street. Our success would not have been possible without the incredible work and support from our partners and collaborators. We are proud to present an overview of our 2020 projects and we look forward to working with you in the new year!

Sincerely,

Yukako Yagi, PhD

Yukako Yagi, PhD

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Multimodal 3-Dimensional (3D) Imaging
Project with Emilia Mason, PhD, Alexei Teplov, Peter Ntiamoah, PhD, Yukako Yagi, PhD

Multimodal imaging enables us to have a greater biological understanding of various cancers and diseases, as well as help to improve diagnostic accuracy. We can overlay images from multiple imaging modalities to provide increased comprehensive insight into the molecular and anatomical features of a model subject. For example, hematoxylin and eosin (H&E) imaging data, is used to identify important features of an organ or tumor. When histopathology images are integrated with micro-computed tomography (micro-CT) we can measure the depth, volume, and additional useful information.

Traditional methods utilize tissue slicing to create glass slides. Previous workflows involved the creation of serial sections (preparing up to 100 consecutive slides from a single tissue block), (ii) applying H&E staining to each slide, (iii) scanning all slides to create histopathology images and (iv) digitally reconstructing the tissue via 3D-reconstruction techniques. However, tissue sectioning often causes certain areas of the tissue to be cut off, resulting in the loss of important anatomical and cellular features. To determine whether whole block imaging (WBI) reveals additional information beyond what can be ascertained from H&E stained slides, we have eliminated the need to create glass slides from our workflow and instead, scan the entire tissue block using a micro-CT scanner.

Whole block imaging (WBI) using micro-CT allows for a 3-dimensional morphometric analysis of tissue in formalin-fixed paraffin-embedded (FFPE) tissue blocks, without any sectioning or loss of sample. Through our current research efforts, we were able to see the glomeruli clearly within a kidney sample through both histology 3D and micro-CT 3D imaging. Any missing areas within the 3D-reconstruction of the H&E serial sectioned slides could be found through micro-CT WBI, and the volume of a single glomerulus could be determined as well. Moreover, utilizing micro-CT imaging on FFPE tissue blocks adds a significant pathologic correlation to the acquired 3D histology data. This type of multimodal imaging analysis will not only help to facilitate new diagnostic discovery and research, but can also help pathologists in performing time-prohibitive tasks in an efficient manner through image correlation and visualization.

Correlating Micro-CT Images of Radical Prostatectomy Specimens with Histopathology
Project with Alexei Teplov, Vincent P. Laudone, MD, Andrew Tracey, MD, S. Joseph Sirintrapun and Yukako Yagi, PhD

When performing a radical prostatectomy, the surgeon must make decisions intra-operatively, balancing the need for negative surgical margins with the goal of preserving the neurovascular bundles that run along the inferolateral margins of the prostate gland. Peripherally located tumors and extra-prostatic extension can pose a challenge, as the presence of a positive surgical margin (PSM) on final pathology independently predicts cancer recurrence. The decision to spare the cavernous nerve risks a positive surgical margin, while a wider resection can sacrifice future potency or continence. Patients at our institution routinely undergo pre-operative multi-parametric MRI in order to aid with surgical planning and assess the safety and feasibility of nerve-sparing. Studies evaluating the ability of a pre-operative multi-parametric MRI to identify extracapsular extension have shown wide variability, with sensitivity ranging from 35% to 78% and specificity of 83% to 92%. There is a need for additional tools to aid surgeons with intra-operative identification of tumor extent, particularly when considering a nerve-sparing dissection.

The use of intra-operative frozen section analysis of the tissue on the prostatectomy specimen adjacent to the neurovascular bundle has shown promise as a tool for real-time histologic monitoring of the oncologic safety of nerve-sparing, but concerns regarding false negatives and the significant institutional resource requirement have prevented widespread implementation. There is a clear need for an accurate, fast, and reproducible tool to aid the surgeon in determining the adequacy of the surgical margin at the time of resection. This study will evaluate the feasibility and accuracy of using micro-computed tomography (micro-CT) to determine the presence of positive surgical margins and extracapsular extension of disease.
Correlating Micro-Computed Tomography Images of Endoscopic Resected Gastrointestinal Specimens with Histopathology

Project with Hirotsugu Sakamoto, MD, PhD, Alexei Teplov, Noboru Kawata, MD, Takashi Ohnishi, PhD, Masao Yoshida, MD, Emine Cesmecio glu, MD, Makoto Nishimura, MD, Peter Nti amoah, PhD, Jinru Shia, MD, and Yukako Yagi, PhD

Pathological evaluation of endoscopically resected superficial lesions in the gastrointestinal lesions based on findings such as tumor invasion depth, lateral/deep margins, lymphovascular invasions, and tumor budding is important for assessing the risk of subsequent recurrence. Micro-computed tomography (micro-CT) can non-destructively provide a three-dimensional (3D) reconstruction of the entire tissue at a high resolution, which enables a good correlation with pathological findings. The aim of this study is to establish the clinical utility of micro-CT to compare images with conventional histology and endoscopic images.

We scanned 8 formalin-fixed paraffin-embedded (FFPE) tissue blocks of ESD specimens (2 gastric cancer and 1 colon cancer) using a custom-built micro-CT scanner. Reconstructed imaging data was visualized and analyzed using commercially available software. The matching cross-section slices between WSIs of H&E-stained slides and micro-CT images, as shown in Figure 1. All reconstructed images with high resolution allowed for the clear visualization of tissue structure and differentiation between tumor and non-tumor tissue. In the micro-CT images, we were able to determine tumor invasion depth, lymphovascular invasion (LVI), and resected margin status by correlating the reconstructed images obtained by micro-CT with those from whole slide imaging (WSI) of hematoxylin and eosin (H&E)-stained slides. In addition, micro-CT images revealed that the actual tumor invasion depth was deeper than the conventional pathological diagnosis in 2 cases of gastric cancer. 3D reconstruction provided a cross-sectional image at any location and increased the ease of identifying the running direction of blood vessels and the horizontal tumor spread pattern, which were difficult to identify using conventional slide images.

Our results showed that WBI by micro-CT has the potential to provide further pathological information related to the treatment strategy after ESD in addition to conventional methods. Further studies in additional cases are needed to confirm this. STAS was identified in both typical carcinoid (TC) and atypical carcinoid (AC). STAS was seen as solid nests extending from the edge of the main tumor invading the lung parenchyma. Near the tumor, many of the STAS nests appeared detached in 2-dimensional glass slides. However, with micro-CT, 3D images revealed that these STAS clusters were connected to each other and frequently to the main tumor with a pattern of tumor islands.

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Correlating Micro-Computed Tomography Images of Endoscopic Resected Gastrointestinal Specimens with Histopathology

Project with Hirotsugu Sakamoto, MD, PhD, Alexei Teplov, Noboru Kawata, MD, Takashi Ohnishi, PhD, Masao Yoshida, MD, Emine Cesmecio glu, MD, Makoto Nishimura, MD, Peter Nti amoah, PhD, Jinru Shia, MD, and Yukako Yagi, PhD

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Application of Micro-Computed Tomography Whole Block Imaging (WBI) for the Evaluation of Endoscopic Submucosal Dissection Specimens

Project with Noboru Kawata, MD, Alexei Teplov, Takashi Ohnishi, PhD, Masao Yoshida, MD and Yukako Yagi, PhD

The precise pathological diagnosis of endoscopic submucosal dissection (ESD) specimens is essential because the criteria for determining subsequent therapeutic steps is the presence or absence of risk factors for lymph node metastasis. The current diagnosis technique involves the evaluation of two-dimensional images of cross-sections of resected specimens, which only evaluates a small part of the tumor. Micro-computed tomography (micro-CT) can provide non-destructive three-dimensional (3D) reconstructed whole block imaging (WBI) at a high resolution. Therefore, this study aimed to determine whether micro-CT can provide further pathological information in addition to conventional diagnosis methods in the evaluation of ESD specimens.

We scanned 8 formalin-fixed paraffin-embedded (FFPE) tissue blocks of ESD specimens (2 gastric cancer and 1 colon cancer) using a custom-built micro-CT scanner. Reconstructed imaging data was visualized and analyzed using commercially available software. The matching cross-section slices between WSIs of H&E-stained slides and micro-CT images, as shown in Figure 1. All reconstructed images with high resolution allowed for the clear visualization of tissue structure and differentiation between tumor and non-tumor tissue. In the micro-CT images, we were able to determine tumor invasion depth, lymphovascular invasion (LVI), and resected margin status by correlating the reconstructed images obtained by micro-CT with those from whole slide imaging (WSI) of hematoxylin and eosin (H&E)-stained slides. In addition, micro-CT images revealed that the actual tumor invasion depth was deeper than the conventional pathological diagnosis in 2 cases of gastric cancer. 3D reconstruction provided a cross-sectional image at any location and increased the ease of identifying the running direction of blood vessels and the horizontal tumor spread pattern, which were difficult to identify using conventional slide images.

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OneDB: A New Dimension to a Multimodality Data Management System

Project with James Relyea, Kareem Ibrahim, Himanshu Joshi, MD, Ziv Frankenstein, PhD, Naohiro Uraoka, MD, PhD, Hussain Shakhawat, PhD, Marc-Henri Jean, Alexie Teplov, Meera Hameed, MD, Yukako Yagi, PhD

The Pathology Digital Imaging Laboratory, led by Dr. Yukako Yagi, is experienced in managing diverse datasets and file formats. The objective of this project is to create a novel database system that allows for the compilation and analysis of various sources of medical information (i.e., pathology, radiology, molecular, whole slide and whole block images, etc.) regardless of its format, source or size. Once completed, this singular database (a.k.a. “OneDB”) will improve not only the efficiency of 2D and 3D image analysis but also ensure diagnostic accuracy.

For example, the team has been developing methods for the reliable automated analysis of FISH, CISH, Micro-CT and histology 3D imaging on a cellular level. Deep learning technologies such as these, will also be deployed utilizing the data housed within OneDB and can be a valuable tool for efficient data/image review, correlation, analysis, and AI application development. This type of tool will not only help to facilitate new diagnostic discovery and research but can also help pathologists in performing time-prohibitive tasks in an efficient manner via data/image correlation and visualization.
An Automated HER2 Quantification Method for Breast Cancer on Both CISH and FISH Whole Slide Images

Project with Hossain Md Shakhawat, PhD, Emine Cesmecioglu, MD, Rene N. Serrette, Emilia Mason, PhD, Willard Wong, MD, Benjamin Stueben, MD, Takashi Inoue, MD, Matthew G. Hanna, MD, Marcia Edelweiss, MD, Edi Brogi, MD, Meera R. Hameed, MD, Dara R. Ross, MD and Yukako Yagi, PhD

HER2 quantification is performed routinely for invasive breast cancer (BC) patients to identify HER2 positive patients suitable for receiving targeted therapy. HER2 positivity is an established prognostic marker associated with more aggressive disease and decreased survival. Fluorescence in situ hybridization (FISH) and chromogenic in situ hybridization (CISH) are FDA approved tests for assessing HER2 gene amplification. In practice, the assessment is performed by pathologists manually counting myriads of signals from FISH slides. Advantages of CISH compared to FISH includes the use of a light microscope, application of morphology, lower cost, and faster slide preparation time; however, manual CISH evaluation is labor-intensive and time-consuming.

In this study, we assess the practicability of an automated HER2 quantification method on CISH whole slide images (WSIs). Primarily, thirty-five cases of invasive BC with prior IHC and/or FISH testing were selected for this study. Subsequent manual assessment by dual-probe CISH was performed and categorized. WSIs were then scanned and regions of interest (ROIs) containing invasive cells were manually annotated and analyzed with Shimaris PACQ v1.3 (in-house application) for automated dual-probe CISH. This application detects a limited number of singular nuclei suitable for quantification using U-net. It scores HER2 and CEP17 signals based on the optical density of RGB values to select only prominent signals and ignore the weak ones. The color of the slide was calibrated using the control prior to scoring. Manual CISH and FISH results were compared to the automated CISH results. Automated CISH was concordant with manual CISH (0.98) and manual FISH (0.96) demonstrating the feasibility of automated HER2 CISH evaluation.

The proposed method was applied on FISH WSIs to further validate the method for FISH quantification. For this study, 4-micron serial section slides were produced in the order of CISH-H&E-FISH. Then, HER2 quantification was performed separately on FISH and CISH slides using the proposed method for 7 invasive BC cases. The quantification results were compared with pathologists’ manual FISH results and found concordant. This ensures the applicability of the proposed automated HER2 quantification method for both CISH and FISH WSIs. One major advantage of having an automated quantification application like the proposed method, which works for both FISH and CISH, is that it allows the laboratories to select the option freely depending on their convenience. Further study in the automated platform could provide clinical evidences and may enable automated analysis for other tumors (i.e., gastric, colon).
Whole Block Imaging (WBI) Utilizing Micro-Computed Tomography (Micro-CT) Reveals Pathological Information Not Detected on Regular Histology: A Pilot Study of Rectal Cancer Resection Specimens

Project with Canan Firat, MD, Hirotsugu Sakamoto, MD, PhD, Alexei Teplov, Emine Cesmecioglu, MD, Yukako Yagi, PhD, and Jinru Shia, MD

The continued improvement in pathological evaluation of rectal cancer resection specimens (including an increasingly more detailed assessment of mesorectum, circumferential margin, tumor deposits [TDs] and lymph nodes [LNs], lymphovascular invasion [LVI], perineural invasion [PNI]) has contributed to the continuing improvement of clinical outcomes. This pilot study aims to explore whether there was an additive utility of using micro-CT WBI to assess the paraffin tissue’s microarchitecture when compared to current approaches in assessing rectal cancer resection specimens.

A total of 138 wholemount-blocks and H&E sections from 9 rectal cancer resections were evaluated using micro-CT WBI. Detailed annotations on the WSIs served as training tools for the recognition of the histologic patterns on WBI. Comparative analyses of WBI and H&E slides were performed. Major histological features (tumor, mucin, LNs, TDs, circumferential margin) were readily recognizable on WBI. WBI recognized viable carcinoma in 7/8 cases (63/64 blocks) that contained viable-tumor and predicted lack of viable carcinoma in 1/1 case (8/8 blocks). Similar accuracy-rates were achieved with mucin, lymph nodes with or without macroscopic metastasis, and TDs. WBI recognized LVI and PNI at a rate of 68% and 28%, respectively. The circumferential margins of each slide were found to be nearly identical for WSI and WBI. Complementing the conventional histology, WBI allowed visualization of the evolution of the histologic features both within the same block and through the entire tumor via contiguous blocks. In some cases, WBI scanned the entire block, which led to additional findings such as the origin of TDs and the other metastatic LNs that could not be confirmed by WSI.

Micro-CT WBI provided a 3D reconstruction that complemented conventional histology. Studies are ongoing to confirm the clinical/biological implication of recent findings that a combination of WSI and WBI could provide a more accurate diagnosis of rectal cancer.

2020 Projects
Peer-Reviewed Publications


Invited Presentations

Yagi Y. Digital & Computational Pathology in Research and Clinical Spaces, Symposium on AI for Biomedical Imaging Across Scales IBM Research. Almaden, San Jose, CA. February, 2020

Yagi Y. Digital Pathology Solution Development for Clinical Applications, The United States and Canadian Academy of Pathology (USCAP), Los Angeles, CA. March, 2020


Yagi Y. Computational Pathology and AI on 3D Imaging, 6th Digital Pathology & AI Congress: USA, Virtual Conference. November, 2020

Yagi Y. Digital & Computational Pathology in Research and Clinical Space, European Society of Digital and Integrative Pathology (ESDIP) Workshop, Digital & Computational Pathology in Research and Clinical Space, Virtual Conference. November, 2020

Thank You

to the Departments of Pathology, Radiology, and Medical Physics, the Josie Robertson Surgical Center, the Warren Alpert Center for Digital and Computational Pathology, Nikon, 3DHistech, Dainippon Seiki, Tokyo Institute of Technology, Dokkyo Medical University Hospital, Shizuoka Cancer Center, Chiba University, and all of our research partners and supporters.

Happy Holidays!

Selected Abstracts

Inoue T, Teplov A, Bekhtman N, Travis WD, Yagi Y. The Roles of Whole Block Imaging with Micro-Computed Tomography in Lung Adenocarcinoma. The United States and Canadian Academy of Pathology (USCAP) 2020, Los Angeles, CA


Uraoka N, Rao M, Zhang Y, Hameed MR, Yagi Y. Toward an Automated Scoring Algorithms of Fluorescence In Situ Hybridization (FISH) on Formalin-Fixed Paraffin-Embedded (FFPE) tissues Using a Confocal Whole Slide Image Scanner and Image Analysis Software. The United States and Canadian Academy of Pathology (USCAP) 2019, Washington, DC


Xu B, Teplov A, Ibrahim K, Inoue T, Stueben BL, Katabi N, Hameed MR, Ghossein RA, Yagi Y. Defining Crucial Pathologic Parameters in Thyroid Neoplasm Using 3D Whole Block Imaging (WBI) with Micro resolution CT scanner (MicroCT): A Proof of Concept. The United States and Canadian Academy of Pathology (USCAP) 2020, Los Angeles, CA

Yagi Y, Ikemura K. Mitosis Detection with Tiny-YOLO. The United States and Canadian Academy of Pathology (USCAP) 2020, Los Angeles, CA
