2018

THE YUKAKO YAGI
Pathology Digital Imaging Laboratory

Memorial Sloan Kettering
Cancer Center™

WELCOME MESSAGE

Dr. Yagi's pathology digital imaging laboratory ("Yagi Lab") at the Josie Robertson Surgical Center provides 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), Yagi lab enhances the assessment and creates opportunities for multidisciplinary applications.

Our current research will further enrich our knowledge of disease by integrating computational pathology data with other specimen-related data (genomics, proteomics, radiographic imaging, etc.). 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.

This year has led to substantial progress in each of our projects, and 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 2018 projects and look forward to working with you again in the New Year.

Yukako Yagi, PhD

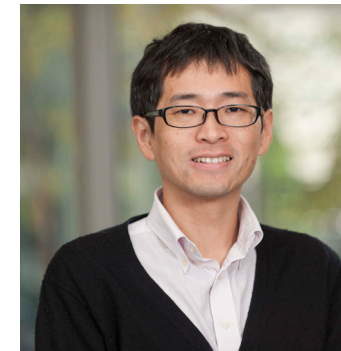## MEET THE TEAM

Alexei Teplov
Sr. Research Technician

Christina M. Virgo, Esq.
Research Program Coordinator

Kareem Ibrahim,
Sr. Application Analyst

Kazuhiro Tabata, MD, PhD
Visiting Investigator

Naohiro Uraoka, MD, PhD
Research Fellow

Patricia Ashby
Pathologists Office Assistant

2018 Projects
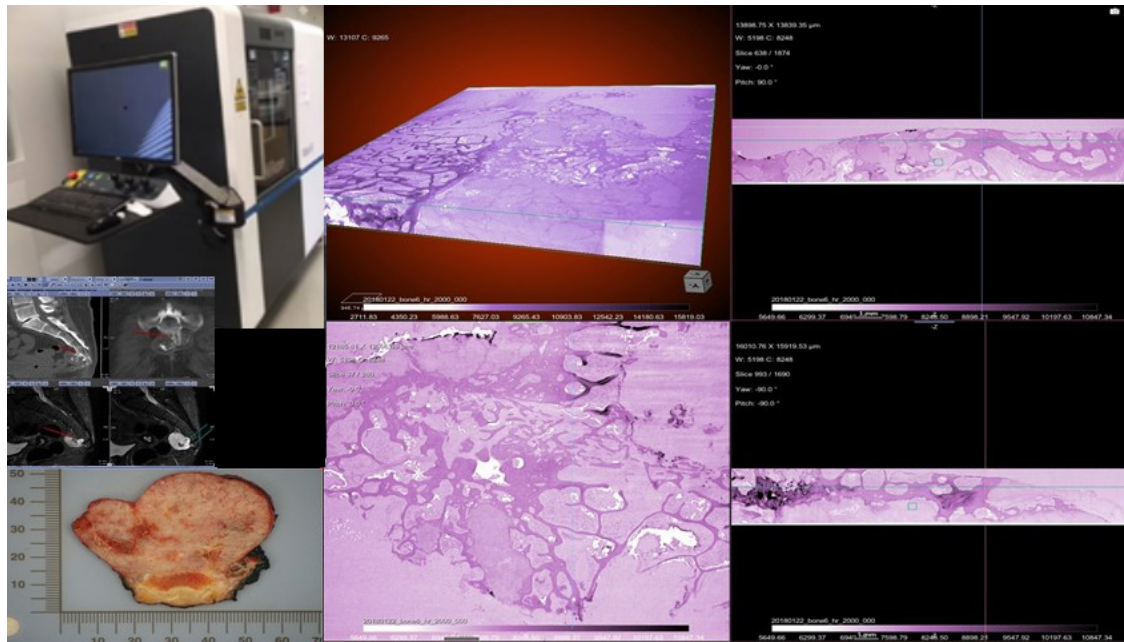
Micro-Computed Tomography in Surgical Pathology

Project with Michael H. Roehrl, MD, PhD, Peter Ntiamoah,PhD, Sahussapont Joseph Sirintrapun, MD, Melissa Murray, DO, Jinru Shia, MD, William D. Travis, MD, Meera R. Hameed, MD, Kazuhiro Tabata, MD, PhD, and Alexei Teplov, Ronald Ghossein, MD, Edi Brogi, MD, Kareem Ibrahim

Micro-computed tomography (micro-CT) is an emerging technology within the biomedical field and holds great promise for imaging pathology specimens for reconstruction, modeling, and three-dimensional (3D) analysis. The capacity of micro-CT to create high-resolution 3D models of ex vivo tissue, therefore, creates a unique opportunity to correlate micro-CT image data with other imaging modalities and bring additional information for diagnosis into the future of Pathology. The objectives of this research are 1) to evaluate micro-CT in tissue specimens in pathology, and 2) to develop a scanning protocol and workflow specific to the tissue specimens.

2018 Updates: Optimization and evaluation paper summarizing results submitted for publication. Results of application presented at national and international conferences.


Correlating Micro-Computed Tomography of Suspicious Breast Lesion (BI-RADS 4 or 5) Core Biopsy Specimens with Histopathology

Project with Project with Hanna Y. Wen, MD, PhD, Melissa P. Murray, DO, Anne Grabenstetter, MD, Kant Matsuda, MD, Edi Brogi, MD, Alexei Teplov, Kareem Ibrahim, Marc-Henri Jean, Naohiro Uraoka, MD, PhD

Micro-CT technology offers high resolution images at the microscopic level (up to a resolution of less than 10 microns) and can allow for the identification of different components such as fibroglandular and adipose tissue in a breast specimen. In this pilot study we will explore the utility of micro-computed tomography in a clinical setting for the evaluation of breast surgical specimens along with histopathologic correlation/validation. Scanning with the micro-CT is completed within 5 minutes, making it suitable for rapid evaluation of fresh surgical specimens without a substantial delay in fixation. The use of micro-CT, could potentially enhance the definition of the tumor spatial distribution, in particular with regard to tumor multifocality. It could also guide selective sampling of the tumor areas, reducing the number of tissue sections/block and workload in the pathology laboratory. We expect this approach to improve the accuracy of breast tissue assessments e.g. lymph nodes status, especially in axillary lymph node dissection.

2018 Updates: Investigation of staining techniques to optimize analysis completed. Project ongoing.

Micro-CT

Micro-CT
Digitally Stained
Tumor cells are detected
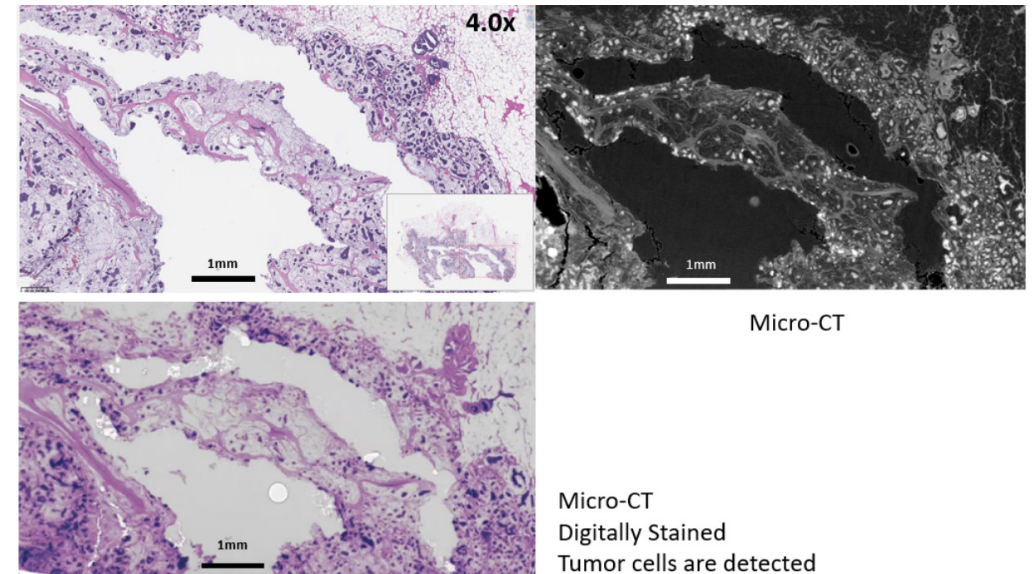
The Roles of Micro-Computed Tomography (CT) in Breast Pathology

Project with Michael Roehrl, MD, PhD, Meera R. Hameed, MD, Kant Matsuda, MD, Melissa Murray, DO, Edi Brogi, MD, Kazuhiro Tabata, MD, PhD, Alexei Teplov, Edi Brogi, MD, Kareem Ibrahim, Marc Henri Jean, Naohiro Uraoka, MD, PhD

It is established that breast cancer spreads by contiguous stromal invasion and also by intraductal proliferation with possible noncontiguous invasion. Intra-operative frozen section evaluation of the surgical margin is advocated by some investigators to accelerate detection of margin involvement and reduce the re-excision rate and local recurrence, but sampling error or skipped lesions may limit the assessment. Micro-computed tomography (micro-CT) enables the study of the three-dimensional structure of the tissue and does not require any tissue sectioning or loss of the sample. We evaluate micro-CT images of fresh breast tissue and lymph nodes and compare the findings with those in micro-CT images of formalin-fixed paraffin embedded (FFPE) tissue blocks with available hematoxylin and eosin (H&E) stained sections.

Fresh tissue | CT of Fresh tissue
CT of Block | HE staining
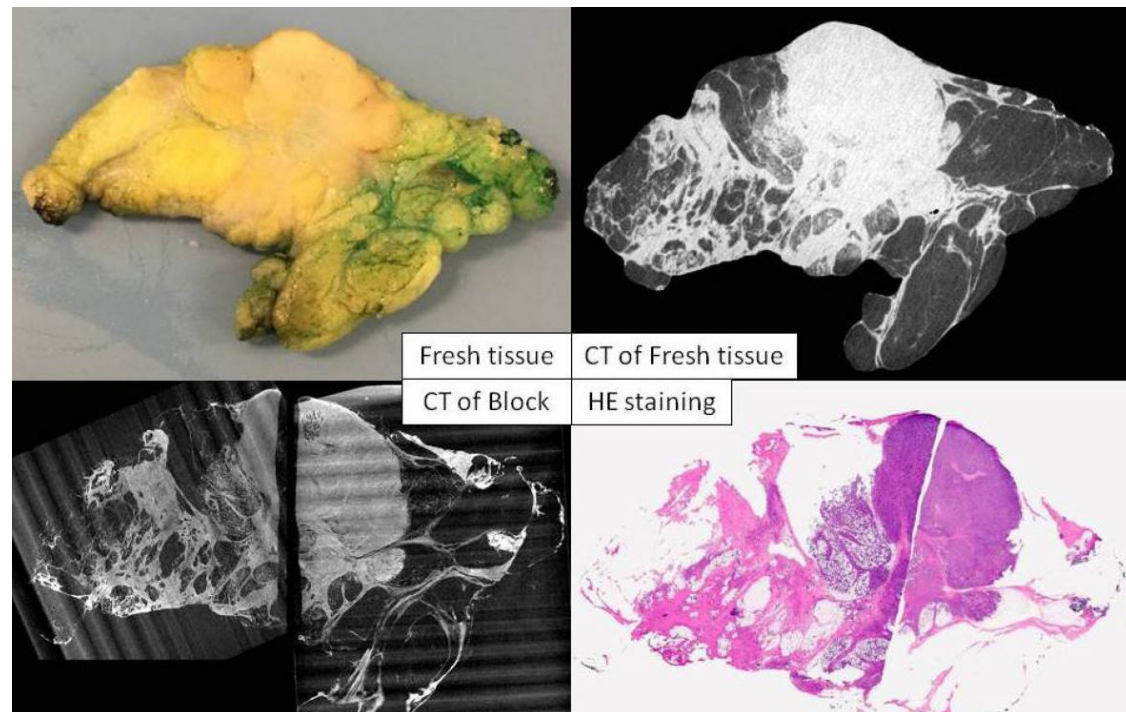
Whole Block Imaging in Prostate Cancer

Project with Sahussapont Joseph Sirintrapun, MD, Anuradha Gopalan, MD, Hikmat A. Al-Ahmadie, MD, Ying-Bei Chen, MD, PhD, Samson W. Fine, MD, Satish K. Tickoo, MD, Victor E. Reuter, MD, Alexei Teplov, Kazuhiro Tabata, MD, PhD

Traditional histologic methods are hindered by glass slide creation through tissue sectioning, staining, and additional scanning. Traditional sectioning microscopy also only provides two-dimensional (2D) image representations of 3D structures. Micro-computed tomography (micro-CT) opens a disruptive imaging opportunity, not only in obviating the need for sectioning and tissue staining but in potentially rendering 3D images useful for new morphologic insights. Recently micro-CT has achieved optimal performance in image acquisition and volume processing. We have applied micro-CT in rendering 3D images of prostate whole mounted paraffin embedded slices.
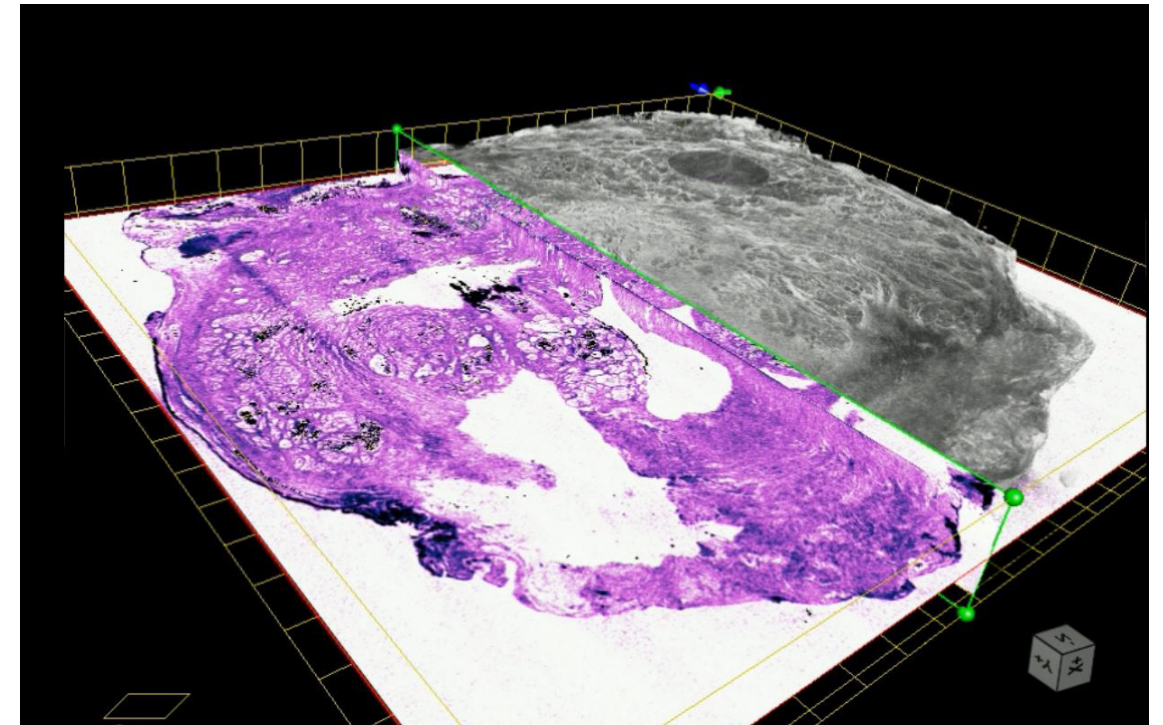
Whole Block Imaging in Bone Tumor

Project with Sinchun Hwang, MD, Meera R. Hameed, MD, Kazuhiro Tabata, MD, PhD, Alexei Teplov

In Orthopedic Pathology, correlation of imaging characteristics to histopathology is important for a meaningful and accurate diagnostic interpretation. This traditional practice has provided many insights on tumor characteristics such as growth pattern, relationship to adjacent normal bone, matrix production, tumor aggressiveness, etc. In Radiology, three-dimensional (3D) reconstruction of digital images is an easily available technique whereas traditional Pathology provides only a 2D image of 3D anatomical structures albeit with much higher resolution than radiological images. Although 3D histological reconstruction is possible, it requires automated sectioning of hundreds of slides with 3D reconstruction using appropriate software. 3D whole block imaging (WBI) by micro-computed tomography provides a new imaging modality to create 3D reconstruction of tissue sections with microscopic level resolution, potentially up to 10x without the need for tissue sectioning.

> **2018 Updates:** We found the micro-CT scanner allows for a deeper analysis than originally anticipated. Our work in 2018 has moved this project to a second phase, with a focus on the use of micro-CT (benefits and limitations) in a clinical setting. We are now able to identify the tumor size and location through WBI, and request diagnostic areas for slide preparation. This development reduces cost and the potential risk of losing tissue that may arise through traditional sectioning methods. We are working to integrate clinical assessments into a more detailed analysis of tumor of the bone.
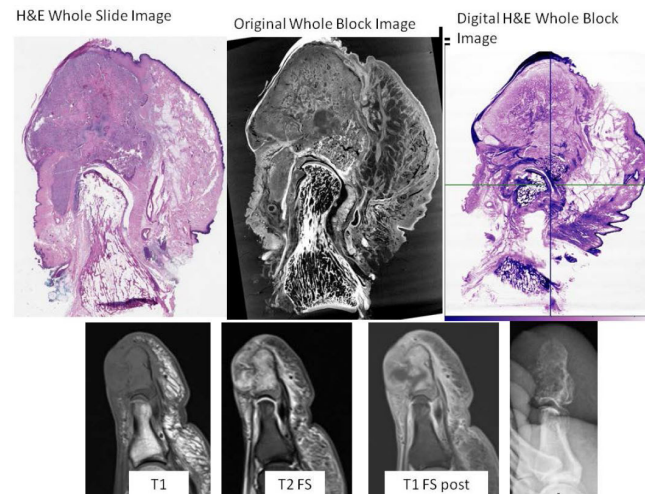

Three-Dimensional Assessment of Spread through Air Spaces in Lung Adenocarcinoma: Insights and Implications

Project with Kazuhiro Tabata, MD, PhD, Natasha Rekhtman, MD, Takashi Eguchi, MD, Joseph Montecalvo, MD, Prasad Adusumilli, MD, Katia Manova, PhD, Meera Hameed, MD and William D. Travis, MD, Rania G. Aly, MD

Tumor spread through air space (STAS) is a newly recognized form of invasion in lung adenocarcinoma and squamous cell carcinoma that growing evidence shows is associated with recurrence and survival. The observation that tumor STAS clusters/nests, or single cells, within air spaces on two-dimensional hematoxylin and eosin slides raised the question: "How could these cells survive within air spaces without a vascular supply?" This, in turn, has led some to speculate that STAS is an artifact. Herein, we perform the high-resolution high-quality three-dimensional reconstruction and visualization of normal lung and tumor in lung adenocarcinoma to investigate the invasive pattern of STAS.

> **2018 Updates:** The concept of three-dimensional immunofluorescence analysis of dynamic vessel co-option of spread through air spaces (STAS) in lung cancer was presented at the 2018 IASLC Conference.
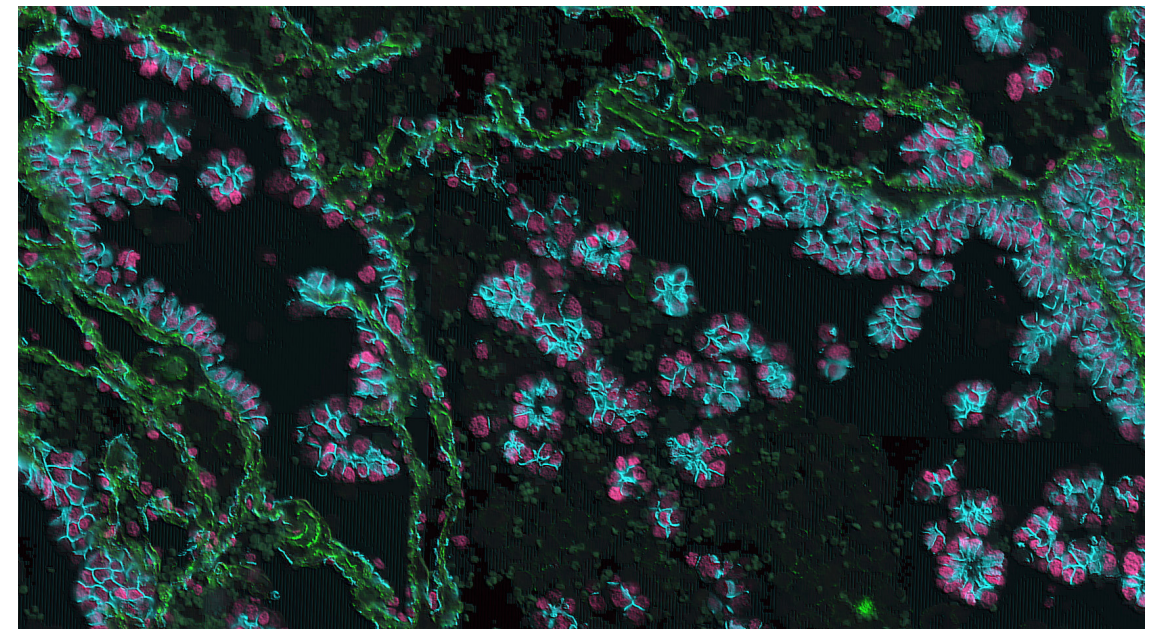
Automated FFPE FISH Signal Scoring using a Confocal Scanner

Project with Meera R. Hameed, MD, Lu Wang, MD, PhD, and Yanming Zhang, MD, Travis Hollmann, MD, PhD, Naohiro Uraoka, MD, PhD

The intention of this project is to fully automate the process of scan data access, nuclear segmentation, fluorescence spot detection and statistical classification of nuclei according to MYC break-apart sorting criteria. Previous analysis was semi-automated, relying on manual methods to generate z-stack image data from confocal scan data and for nuclear segmentation using CaseViewer and Imaris, respectively. The automated system utilizes the OpenCV computer vision framework and a skimage image processing library in conjunction with the python programming language to segment and classify nuclei, count spots, and generate summary statistics based on the clinical scoring system. Reports will be automatically generated by the system and statistics will persist in a relational database management system dedicated to the project.

2018 Updates: Additional cases added for analysis and validation to prepare for move to automation.
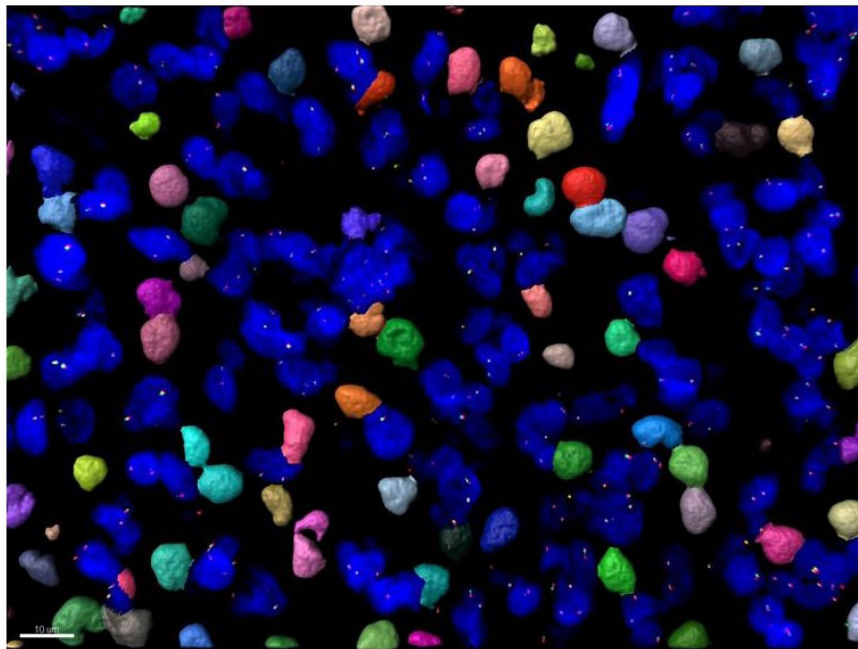

Chromogenic in situ Hybridization and Digital Pathology

Project with Dara S. Ross, MD, Marcia Edelweiss, MD, Edi Brogi, MD, Rene Serrette, Handy Oen, Meera R. Hameed, MD, and Matthew G. Hanna, MD

Human epidermal growth factor receptor 2 (HER2) testing is vital to predicting potential responsiveness for HER2-targeted therapies in breast cancer. To determine HER2 amplification status, chromogenic in situ hybridization (CISH), which utilizes peroxidase or alkaline phosphatase reactions and is visualized by standard bright-field microscopy, has been developed as an alternative method to fluorescence in situ hybridization (FISH). CISH is evaluated by the manual counting of more than 20 cancer nuclei, according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guideline. Furthermore, the evaluation process of CISH is time-consuming. The purpose of this study is to establish an in-house image analysis tool for the automated CISH scoring that is clinically applicable.

The methodology of this study is straight-forward; archival CISH slides from surgically resected breast cancer cases are digitized by a whole slide image scanner. Several regions of interest are selected to create algorithms for automated scoring. The results are then compared to the scores from manual counting.
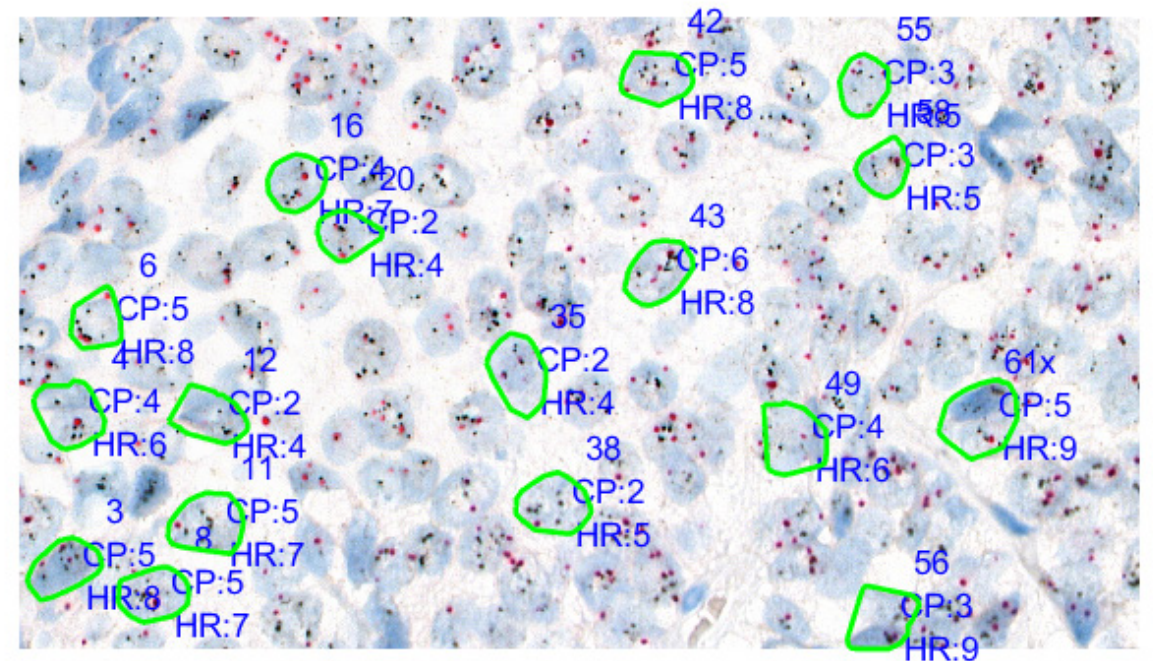
Cytology Evaluation Studies

Project with Oscar Lin, MD, PhD, and Naohiro Uraoka, MD, PhD,

Recently, our Digital Pathology System received approval from the FDA. This system enables us to make pathological diagnoses using whole slide imaging techniques. General histological slides are sectioned with 4-5um in thickness, which is enough to scan with single layer scanning. However, cytology specimens vary in thickness and size when compared to Histology. This is because cytology specimens include cell clusters comprised of more than 10um in thickness. We are seeing a variation in single layer scanning for cytology slides based on the Institution. Thus, multilayer scanning is necessary for the accurate diagnosis of cytology specimens.

If we increase the number of layers we scan, scanning time and file size will be increased. We have to find a balance between the value of having layers versus scanning time/file size for analysis and diagnosis.

The purpose of this project is to optimize the scanning protocol per type in cytology, such as smear, liquid based thin prep, cell block, etc. We will focus on the number of layers, the total distance and the step size to establish scanning parameters. We will investigate image quality, scanning duration and file size as well. Then we will validate our optimized protocol for diagnostic purpose
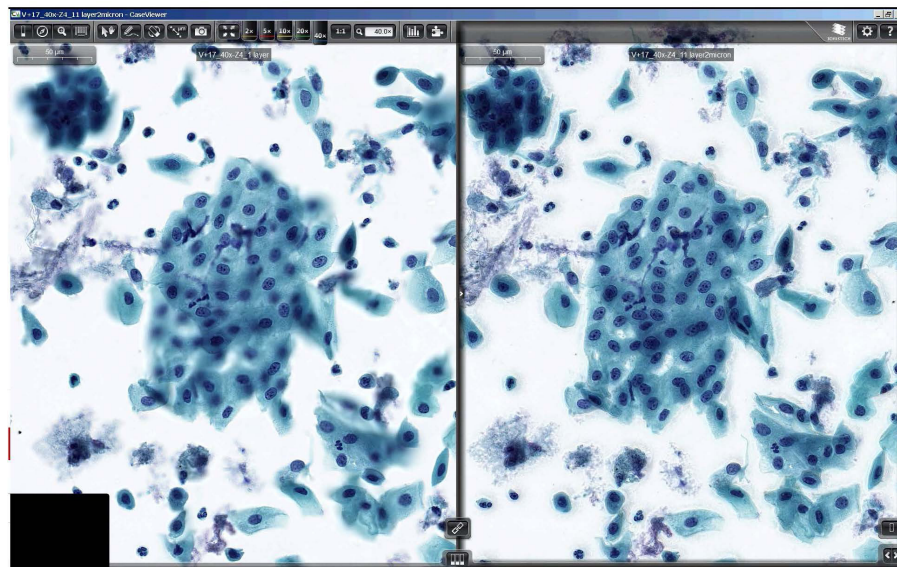
1 Layer   11 layers Extended focus

## Technical and Clinical Standardization for Digital Pathology

The Yukako Yagi Lab

Working to develop small but useful technologies in digital pathology to enhance workflows.

Whole Slide Image Based On-Line Conferencing

Project with Kazuhiro Tabata, MD, PhD, and Naohiro Uraoka, MD, PhD,

Digital pathology systems facilitate telepathology, including consultation. After testing the telepathology web conference capabilities of multiple vendors, we analyzed the merits and demerits of each. Our NDP server 3.0 software (Hamamatsu photonics, Hamamatsu, Shizuoka, Japan) is a digital pathology system that makes in-network consultation and conferences easy and accessible in both clinical and educational settings.
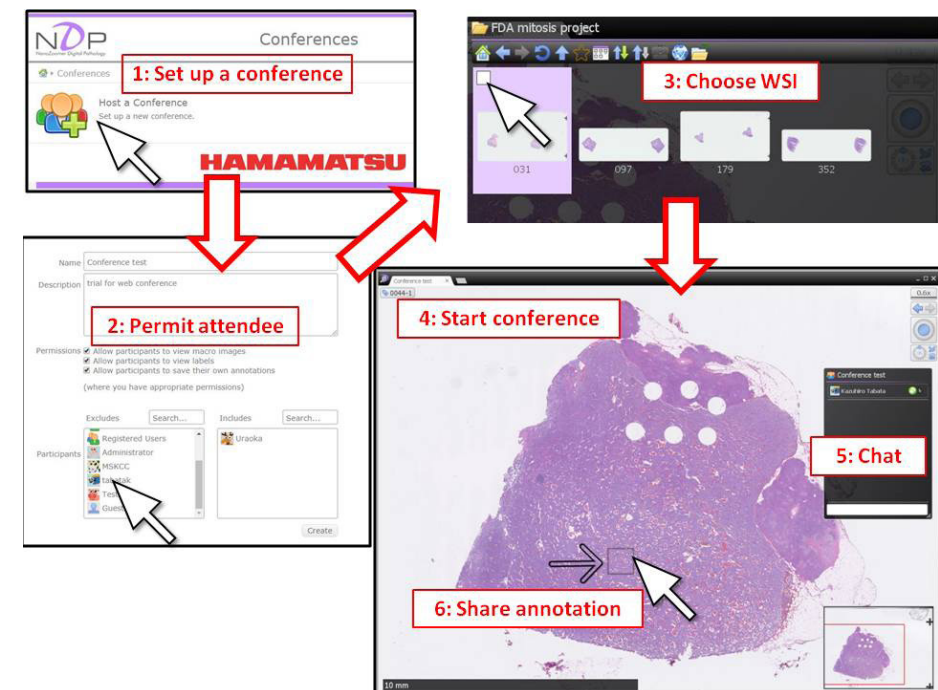
Mitotic Counting and Classification Study

Project with Brandon D. Gallas, PhD, Jamal K. Benhamida, MD, Qi Gong, Matthew G. Hanna, MD, Partha Mitra, PhD, Sahussapont Joseph Sirintrapun, MD, Meera R. Hameed, MD, David S. Klimstra, MD, Naohiro Uraoka, MD, PhD, Kazuhiro Tabata, MD, PhD, Katsura Emoto, MD, Rania Aly, MD

Supervised image analysis algorithms are only as good as the ground-truth on which they are trained. The most practical ground-truth for training an algorithm is a pathologist's assessment on whole slide images (WSIs). Inter-observer variability may affect the reliability of the algorithm. Annotations from WSIs are subject to other limitations such as the inability to focus on nearby planes of a section (as may occur on a microscope). In this work, we conducted a preliminary feature analysis study detecting mitoses with WSIs and with a microscope. This study provides candidate mitosis for a larger study to be conducted on a 14-head microscope. Data from the larger study will be used to evaluate the performance of an automated mitosis detection algorithm. Detecting and quantifying mitosis is an important pathology task when evaluating tumors in many organs; it is also challenging and burdensome to pathologists. Because such a task is likely to be impacted by scan quality, we also investigate its suitability for evaluating image quality.

2018 Updates: Paper submitted for publication and results presented at International conferences.
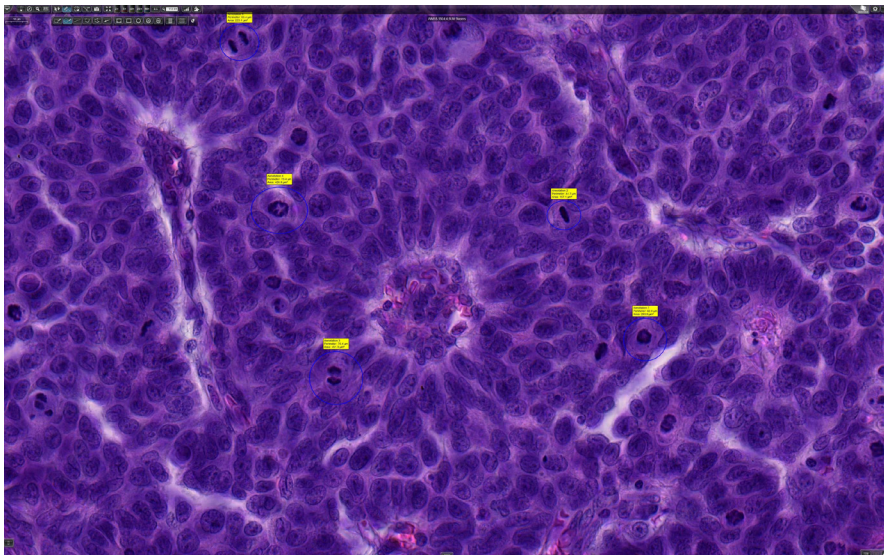
Digital Pathology External Quality Assurance (EQA) Project

Project with Naohiro Uraoka, MD, PhD, Marc-Henri Jean, Chad M. Vanderbilt, MD, Matthew G. Hanna, MD

The objective of this project is to set up a digital pathology external quality assurance scheme to evaluate the quality of image-based counting/scoring mechanism of institutions worldwide. Our first application is for pancreatic neuroendocrine neoplasm cases at MSKCC.
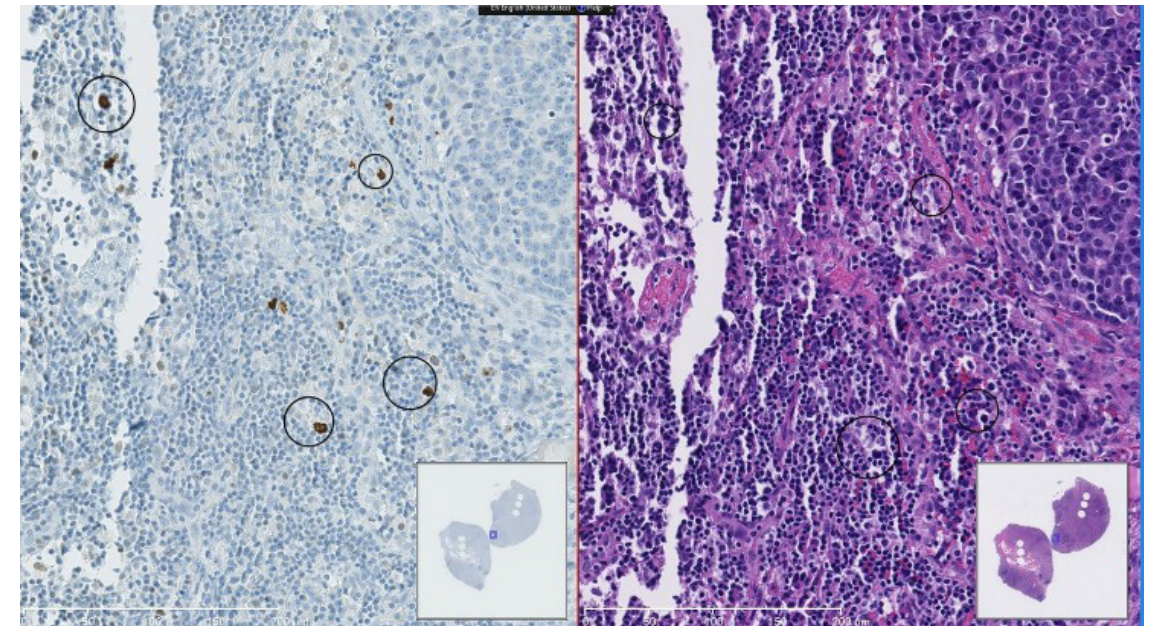
2019 UPCOMING PROJECTS

Warren Alpert Center Research Fund Award Winners (2018)

We excited to include the winners of the 2018 Warren Alpert Center Research Fund Award Warren Alpert Center Research Fund Award. These projects will take our current research to the next phase: clinical application.

MicroCT and Histology 3D Analysis in Rectal Cancer
Exploring the feasibility of utilizing micro-CT to detect clinically relevant pathological alterations in paraffin blocks of rectal cancer specimens.
Jinru Shia, MD, Efsevia Vakiani, MD, PhD, Julio Garcia-Aguilar, MD, PhD, Marc J. Gollub, MD, FACR

MicroCT and Histology 3D Analysis in the Thyroid

3D Imaging of Papillae, Capsular Invasion and Vascular Invasion in Encapsulated Follicular Cell Derived Thyroid Neoplasms
Ronald Ghossein, MD, Nora Katabi MD

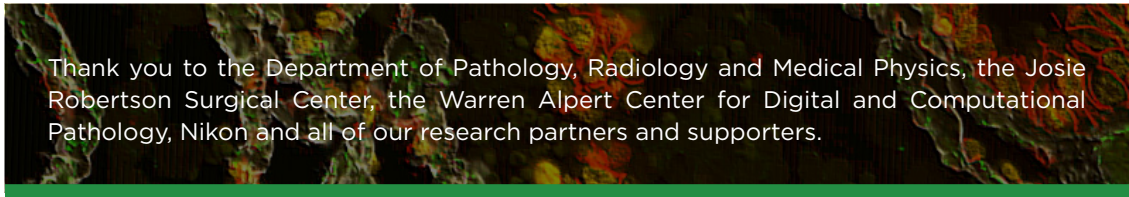### FISH application to Classical Hodgkin Lymphoma (cHL)
Precise detection of PDL1 and PDL2 Amplification in Classical Hodgkins Lymphoma using Confocal Microscope and Simultaneous Visualization of Immunophenotypes and FISH Signals
Yanming Zhang MD, Umut Aypar, MD, Travis Hollmann, MD, PhD, Ahmet Dogan, MD, Meera Hameed, MD

CISH application to Breast Cancer

Assessment of HER2 Amplification Status in Invasive Breast Cancer using Bright-Field In Situ Hybridization and Digital Pathology
Dara S. Ross, MD, Marcia Edelweiss, MD, Edi Brogi, MD, Rene Serrette, Handy Oen, Meera R. Hameed, MD, and Matthew G. Hanna, MD

Thank you to the Department of Pathology, Radiology and Medical Physics, the Josie Robertson Surgical Center, the Warren Alpert Center for Digital and Computational Pathology, Nikon and all of our research partners and supporters.