Confocal Fluorescence Microscopy Platform Suitable for Rapid Evaluation of Small Fragments of Tissue in Surgical Pathology Practice

Savitri Krishnamurthy, MD; Kechen Ban, PhD; Kenna Shaw, PhD; Gordon Mills, MD, PhD; Rahul Sheth, MD; Alda Tam, MD; Sanjay Gupta, MD; Sharjeel Sabir, MD

**Context.**—Rapid advances in the fields of biophotonics, computer science, and instrumentation have allowed for high-resolution imaging of biologic tissues.

**Objective.**—To evaluate the quality of images from an optimized confocal fluorescence microscopy (CFM) platform for rapid evaluation of small fragments of tissue, compared with hematoxylin-eosin staining.

**Design.**—Tissue fragments (up to 1.0 × 0.3 cm) were stained with 0.6 mM acridine orange for 60 seconds and imaged using a CFM platform at 488-nm and 785-nm wavelength. The imaged tissues were then fixed in formalin and processed to generate hematoxylin-eosin–stained tissue sections. The quality of CFM images was scored on a scale of 0 to 3 on the basis of the percentage of the CFM images with recognizable tissue architecture (0, 0%; 1, <20%; 2, 20%–50%; 3, >50%). The diagnoses made using CFM images were compared with those made using histopathologic analysis of the hematoxylin-eosin–stained tissue sections.

**Results.**—We imaged 118 tissue fragments obtained from 40 breast, 23 lung, 39 kidney, and 16 liver surgical excision specimens. We acquired CFM images in 2 to 3 minutes; 95.8% (113 of 118) of images showed a quality score of 3, and 4.2% (5 of 118) had a score of 2. We achieved a sensitivity of 95.5%, specificity of 97.3%, positive predictive value of 95.5%, and negative predictive value of 97.3%.

**Conclusions.**—Our results demonstrate the suitability of the CFM platform for rapid and accurate evaluation of small tissue fragments in surgical pathology practice.

(Arch Pathol Lab Med. 2019;143:305–313; doi: 10.5858/arpa.2018-0352-OA)
Touch preparation is another technique that is more commonly used currently for rapid evaluation of CNB specimens. Although the overall sensitivity of touch preparation for evaluating the quality of CNB specimens is around 90%, this method does not allow accurate evaluation of the tumor cellularity, which is necessary for determining the adequacy of the CNB specimen for the basic diagnosis, as well as for other potential ancillary studies, such as immunohistochemistry and molecular testing. Also, the process of touching the tissue on the glass slide can result in distortion and loss of tissue from the CNB specimen, resulting in less-than-optimal preservation of the CNB specimen for subsequent conventional histopathologic examination.

Therefore, ex vivo tissue imaging has a potential role for real-time evaluation of CNB and small biopsy specimens, ensuring procurement of adequate tissue for making the histologic diagnosis and for successful performance of the appropriate ancillary testing.

The feasibility of using CFM for ex vivo imaging of tissues encountered in surgical pathology practice was reported by us recently. Because of the promising and potentially useful application of ex vivo tissue imaging for rapid evaluation of image-guided CNB specimens, we chose to optimize the CFM platform for evaluating small fragments of tissue cut from surgical specimens to resemble CNB specimens. This would enable us to subsequently use the platform to initiate prospective clinical studies for rapid tissue evaluation of interventional radiology-guided CNB specimens in real time at the bedside in the radiology suite.

In the current pilot study, we tested the performance of the CFM platform optimized for rapid ex vivo imaging of small fragments of tissue resembling interventional radiology-guided, 9- to 18-gauge CNB specimens. The primary objectives of our study were to demonstrate the ability of the optimized CFM platform to generate grayscale and pseudocolored images for tissue qualification, as well as to evaluate the quality of the images and the sensitivity and specificity of the diagnosis that could be made using these images compared with the gold standard of conventional histopathologic examination of hematoxylin and eosin (H&E)–stained tissue sections.

MATERIALS AND METHODS

This prospective study was conducted at The University of Texas MD Anderson Cancer Center (Houston, Texas) using an institutional review board–approved protocol that allowed use of residual tissues obtained from surgical specimens with waiver of informed consent. The tissue fragments were received fresh from the surgical pathology laboratory after completion of intraoperative assessment and were cut into fragments measuring from 0.6 to 1.0 cm in length and 0.2 to 0.3 cm in thickness to mimic CNBs. The tissue was placed in a Petri dish, moistened with phosphate-buffered saline, placed in a biohazard plastic bag, and brought to the radiology suite for imaging. We obtained 118 small tissue fragments of normal and tumor tissue from surgical resections of breast, lung, liver, and kidney for our imaging study.

Imaging was performed using a commercially available confocal scanning fluorescence microscope (RS-G4, Caliber ID, Inc, Rochester, New York). The system measures 18.25 × 15.5 × 16.0 inches and includes 2 diode lasers (785 and 488 nm, respectively) for reflectance and fluorescence imaging, a 550-nm bandpass filter, maximum illumination power of 5 mW, and a x40 oil immersion objective lens with a numeric aperture of 0.9. At these settings, the lateral resolution was 1.0 μm; axial resolution was less than 5.0 μm. Images were acquired at a framerate of 6 frames per second.

The CFM was operated in the radiology suite adjacent to the rooms housing the computed tomography, ultrasound, and magnetic resonance imaging machines. Specimens were kept moist in isotonic phosphate-buffered saline (pH 7.4) prior to imaging. The fresh tissue specimens were stained with 0.6 mM acridine orange for 1 minute to stain the nucleus and create the necessary contrast between the nucleus and cytoplasm for recognition of tissue architecture. Excess dye was dabbed from the stained tissue with gauze prior to imaging. We designed a custom tissue holder for the CFM platform for imaging small strips of tissue that minimizes tissue motion artifact and permits imaging of multiple specimens during a single session. The acridine orange–stained tissue fragment was placed in the tissue holder and covered with optical glass to prevent drying.

The tissue holder was mounted on a motorized stage, and the surface of the tissue was located visually by manually adjusting the position of the lens. Imaging quality was optimized by adjusting imaging depth or plane of focus, and laser power and detector gain were adjusted to optimize brightness and contrast. Mosaics were captured in an automated fashion by scanning the surface of the specimen from one end to the other and stitching the images together to form a larger field of view. The resulting composite image measured up to 2.0 cm in greatest diameter. Composite images were captured from the surface of the tissue. The specimens were scanned in reflectance and fluorescence modes simultaneously and could be viewed as grayscale or fluorescence pseudocolored by viewing each detector channel independently or together. Image mosaics were acquired within 2 to 3 minutes of initiating scanning, depending on the size and number of the specimens. We captured CFM images at 1 depth alone if the quality of the acquired image was good. However, if the quality of the images obtained from the surface was less than optimal we acquired the images at 1 or more deeper levels of the tissue so as to obtain a better quality of the image for interpretation. After completion of imaging, the tissue was immediately fixed in 10% neutral-buffered formalin, subjected to routine processing, and embedded in paraffin wax to generate tissue blocks. The formalin-fixed and paraffin-embedded tissue blocks were cut at 5-μm thickness and stained by the H&E method for conventional histopathologic examination.

The CFM images were viewed and interpreted either at the microscope or on a remote computer using the Teamviewer software after connecting with a unique password generated in the platform at the site of imaging. Custom software (RS-G4) was used to view the mosaics at varying magnifications using mouse-activated zoom and pan features, allowing for basic categorization and interpretation of the tissue to arrive at a histologic diagnosis within a few minutes. The CFM images were interpreted first blinded and separate from the findings in the H&E tissue sections of the imaged tissue. Composite grayscale and fluorescence mosaics were examined in detail at different magnifications, and tissue was categorized as benign, atypical, suspicious, and malignant, and subsequently for a specific histologic diagnosis. Additionally, the mosaics were pseudocolored blue (resembling Toluidine blue–stained histopathology) and also analyzed and categorized according to the same criteria. The CFM diagnosis of the tissue was compared with the conventional histopathologic examination of the H&E tissue sections obtained from the imaged tissue. The quality of the images was graded semiquantitatively into 4 categories based on the extent of the image that demonstrated the tissue architecture clearly, including optimal contrast between the nucleus and cytoplasm, thereby allowing accurate interpretation of the tissue: 0, 0% of image interpretable; 1, less than 20% of the image optimal for interpretation; 2, 20% to 50% of the image optimal for interpretation; and 3, more than 50% of the image optimal for interpretation. The sensitivity and specificity of making the pathologic diagnosis based on the CFM images using both grayscale and pseudocolored images were determined by comparing it to the diagnosis made on H&E-stained tissue sections of the same imaged tissue as the gold standard. We also tested the feasibility of viewing and interpreting the CFM images not only at the site of acquisition of the images in the radiology suite but also remotely in the pathologist’s office.
RESULTS

We imaged 118 tissue fragments, including 40 specimens obtained from surgical resection of breast tissue, 39 from resection of kidney tissue, 23 from resection of lung tissue, and 16 from resection of liver tissue. The CFM images were found to be of good quality; more than 50% of the images exhibited optimal contrast between the nucleus and cytoplasm with adequate sharpness and resolution needed for interpretation in 95.8% (113 of 118) of specimens. We found that 4.2% (5 of 118) of the specimens yielded CFM images with 20% to 50% of the image exhibiting features adequate for interpretation. None of the CFM images were of poor quality, with less than 20% of the image allowing recognition of the tissue architecture. Focal areas in the mosaic of CFM images showed a lack of sharpness, and some areas were dark, implying that light did not penetrate those areas of the tissue. Fortunately, these problems were noted only in small foci in the CFM images. Also, in some cases the presence of necrosis and hemorrhage precluded staining of the tissue, and therefore the tissue architecture could not be appreciated in those areas.

Overall, all CFM grayscale images had at least some areas with the required resolution, thereby allowing interpretation and recognition of tissue architecture and cytomorphologic features. The nuclear staining from the acridine orange created the necessary contrast between the nuclear and cytoplasmic areas of the cell, thereby aiding in the recognition of the tissue architecture. Although the grayscale images were sharper than the pseudocolored images, the staining of all nuclei in the tissue, including stromal cells, endothelial cells, and inflammatory cells, made the recognition and distinction of the lesional cells from other cells in the background difficult. On the other hand, the pseudocolored images were less sharp than the grayscale images, but they allowed for easy recognition of the lesional cells compared with those in the background. Therefore, we found both images to be very useful for accurate evaluation of the specimen.

Conventional histopathologic examination of the 40 H&E-stained breast tissue sections showed that 23 were benign or normal breast tissue, and 17 were malignant. The histopathologic diagnoses of the malignant breast tissues included 2 ductal carcinoma in situ, 2 invasive lobular carcinoma, 10 invasive ductal carcinoma, 1 mixed ductal and lobular carcinoma, 1 invasive micropapillary carcinoma, and 1 metastatic carcinoma. The grayscale and pseudocolored CFM images of the breast tissue fragments showed that 25 were benign and 15 were malignant, in contrast to 23 benign and 17 malignant diagnoses with the H&E-stained tissue sections. The 2 specimens interpreted as benign on the CFM images contained small foci of invasive ductal carcinoma or invasive lobular carcinoma on the H&E-stained tissue sections. The small focus of invasive lobular carcinoma was missed on the CFM images, resulting in a true false-negative interpretation. However, the small focus of invasive ductal carcinoma found on the H&E-stained tissue section was absent in the CFM image, appearing only with deeper sectioning of the formalin-fixed, paraffin-embedded tissue block. The specific diagnosis for the remaining 15 malignant specimens matched exactly between the CFM images and the H&E-stained tissue sections, including 2 ductal carcinoma in situ, 1 invasive lobular carcinoma, 10 invasive ductal carcinoma, 1 mixed ductal and lobular carcinoma, 1 invasive micropapillary carcinoma, and 1 metastatic carcinoma.

We imaged 39 kidney tissue fragments, among which H&E-stained tissue sections showed 20 normal specimens, 2 benign tumor (including oncocytoma) specimens, 15 malignant specimens, and 2 specimens that were extensively necrotic without any viable tissue. The 15 malignant specimens included 10 renal cell carcinoma, clear cell type; 3 renal cell carcinoma, papillary type; and 2 chromophobe renal cell carcinoma. The categorization of the kidney tissue fragments into these diagnostic categories and the specific diagnoses were entirely concordant between the CFM images and the H&E-stained tissue sections. The 2 specimens with entirely necrotic tissue were recognized accurately as such on the CFM images.

Among the 23 lung tissue fragments, 14 were normal lung tissue and 9 were malignant, according to histopathologic examination of the H&E-stained tissue sections. The malignant specimens included 2 squamous cell carcinoma and 7 adenocarcinoma of the lung. Although the 9 malignant specimens were interpreted accurately on the CFM images as 2 squamous cell carcinoma and 7 adenocarcinoma, only 12 of the 14 normal lung tissue specimens were accurately recognized as such on the CFM images. One specimen with prominent type 2 pneumocytes was wrongly interpreted as a small focus of adenocarcinoma, and in the other, a small focus of dilated alveolar spaces filled with macrophages was mistaken as squamous cell carcinoma. Therefore, 2 specimens were falsely categorized as malignant. Similar to the excellent results that we achieved with CFM diagnosis of kidney tissue, the diagnoses made on CFM images of the 16 liver tissue fragments matched exactly with the histopathologic diagnoses. The 16 liver tissue fragments were accurately categorized as 8 normal liver specimens, 7 adenocarcinoma, and 1 hepatocellular carcinoma.

The overall sensitivity of the diagnoses made using the CFM images, compared with the histopathologic diagnoses, was 95.5%; the specificity was 97.3%; the positive predictive value was 95.5%; the negative predictive value was 97.3%; and the accuracy was 96.6%. A summary of the CFM diagnoses compared with the histopathologic diagnoses is shown in the Table. Figure 1 shows representative grayscale (Figure 1, A, D, G, and J) and pseudocolored CFM (Figure 1, B, E, H, and K) images matched with the H&E-stained tissue sections of normal breast, lung, liver, and kidney tissues (Figure 1, C, F, I, and L) that were correctly recognized as such in the study. Figure 2 shows representative examples of correctly identified malignant tumors, including the grayscale (Figure 2, A, D, G, and J) and pseudocolored CFM (Figure 2, B, E, H, and K) images with the matched H&E-stained tissue sections (Figure 2, C, F, I, and L). Figure 3 shows the CFM images (Figure 3, A and D), grayscale CFM images, and Figure 2, B and E, pseudocolored CFM images) and the corresponding H&E-stained tissue sections of the 2 normal lung tissue specimens that were incorrectly diagnosed as adenocarcinoma (Figure 3, C) and squamous cell carcinoma (Figure 3, F) and the breast tissue with invasive ductal carcinoma (Figure 3, I, H&E-stained tissue sections) and invasive lobular carcinoma (Figure 3, L, H&E-stained tissue sections) that were not recognized on the CFM images (Figure 3, G and J, grayscale CFM images, and Figure 2, H and K, pseudocolored CFM images).
clinical practice. The results of our study clearly demonstrate the first step to demonstrate the utility of this platform for radiology–guided CNB specimens in the radiology suite as subsequently for rapid bedside evaluation of interventional platform and, if performance was suitable, to use it acquisition and remotely. Our intent was to test the be made; and viewing of the CFM images both at the site of entire tissue that allow an accurate pathologic diagnosis to

of high-quality grayscale and pseudocolored images of the such as CNB specimens; rapid (2- to 3-minute) acquisition for easy placement and imaging of small fragments of tissue,

optimized CFM platform used in the current study, in

obtained from different solid organs. The capabilities of the optimized a platform for imaging small fragments of tissues into surgical pathology practice. The potential applications for the field of surgical pathology include rapid bedside tissue qualification of small tissue fragments, such as CNB and endoscopic biopsy specimens, the platform can be further improved to address some of the shortcomings. Achieving optimal compression of the tissue fragments, which are not uniform in thickness, resulted in patchy dark areas in grayscale or whitish areas in pseudocolored images where light did not penetrate adequately. However, such areas were generally small and interspersed amid areas with good visualization of the tissue architecture with the needed contrast between the nucleus and cytoplasm, thereby enabling accurate interpretation. Another problem we observed was the presence of necrosis or hemorrhage when the acridine orange did not stain the tissue, resulting in an inability to visualize the tissue architecture. Also, the resolution could be improved, further enabling better recognition of the nuclear characteristics of the tissue.

Although we achieved our objective of demonstrating the ease and suitability of the optimized CFM platform for real-time tissue qualification of small tissue fragments, such as CNB and endoscopic biopsy specimens, the platform can be further improved to address some of the shortcomings. Achieving optimal compression of the tissue fragments, which are not uniform in thickness, resulted in patchy dark areas in grayscale or whitish areas in pseudocolored images where light did not penetrate adequately. However, such areas were generally small and interspersed amid areas with good visualization of the tissue architecture with the needed contrast between the nucleus and cytoplasm, thereby enabling accurate interpretation. Another problem we observed was the presence of necrosis or hemorrhage when the acridine orange did not stain the tissue, resulting in an inability to visualize the tissue architecture. Also, the resolution could be improved, further enabling better recognition of the nuclear characteristics of the tissue.

DISCUSSION

Ex vivo optical imaging can enable visualization of tissues with minimal or no preparation and without destroying or losing tissue. Therefore, this technology, if optimized for rapid tissue qualification, has the potential for incorporation into surgical pathology practice. The potential applications of this technology for the field of surgical pathology include rapid bedside tissue qualification of specimens such as CNBs or endoscopic biopsies, intraoperative assessment of margins of surgical resections, or rapid examination of any small fragments that are sent by the surgeons for frozen section processing. Ex vivo optical imaging techniques can also be used to identify tissue representative of the lesion for sampling, genomic testing, or biobanking.

We pursued ex vivo optical imaging using CFM and optimized a platform for imaging small fragments of tissues obtained from different solid organs. The capabilities of the optimized CFM platform used in the current study, in comparison with our previous report, include a tissue holder for easy placement and imaging of small fragments of tissue, such as CNB specimens; rapid (2- to 3-minute) acquisition of high-quality grayscale and pseudocolored images of the entire tissue that allow an accurate pathologic diagnosis to be made; and viewing of the CFM images both at the site of acquisition and remotely. Our intent was to test the platform and, if performance was suitable, to use it subsequently for rapid bedside evaluation of interventional radiology–guided CNB specimens in the radiology suite as the first step to demonstrate the utility of this platform for clinical practice. The results of our study clearly demonstrate the suitability of the CFM platform for rapid evaluation of small tissues, given the ease of acquiring grayscale and pseudocolored images within a few minutes of placing the acridine orange–stained tissue for imaging and the superb diagnostic ability, as exemplified by the sensitivity and specificity that we achieved in our single-pathologist study. Although the quality of the images varied between the specimens, all images were found to be acceptable for interpretation compared with the gold standard of conventional histopathologic examination of H&E-stained tissue sections. The ability to visualize and interpret the images in real time at the time of acquisition, either at the site of tissue imaging or remotely, is a useful feature for pathology practice. The size and portability of the CFM platform used in our study is an added advantage that can allow acquisition of images from procured tissues in real time in any clinic or radiology suite where biopsies are performed.

Although we achieved our objective of demonstrating the ease and suitability of the optimized CFM platform for real-time tissue qualification of small tissue fragments, such as CNB and endoscopic biopsy specimens, the platform can be further improved to address some of the shortcomings. Achieving optimal compression of the tissue fragments, which are not uniform in thickness, resulted in patchy dark areas in grayscale or whitish areas in pseudocolored images where light did not penetrate adequately. However, such areas were generally small and interspersed amid areas with good visualization of the tissue architecture with the needed contrast between the nucleus and cytoplasm, thereby enabling accurate interpretation. Another problem we observed was the presence of necrosis or hemorrhage when the acridine orange did not stain the tissue, resulting in an inability to visualize the tissue architecture. Also, the resolution could be improved, further enabling better recognition of the nuclear characteristics of the tissue.

The In Vivo Microscopy Committee of the College of American Pathologists, which includes pathologists with clinical experience in in vivo microscopy and research experience with in vivo microscopy and ex vivo microscopy (EVM), recently came up with a list of functional requirements to facilitate the development of EVM platforms for potential applications in pathology practice. These requirements were benchmarked to existing standard technology for immediate evaluation of tissues in surgical pathology practice, including frozen section, touch preparation, and cytologic smear methods. The committee suggested that the EVM platform for evaluation of CNB specimens should be easy to use and require minimal training to operate, and the platform should also be safe, portable, and fast, requiring no more than 10 minutes to acquire the images. Most importantly, the platform should have excellent diagnostic capability, with a positive predictive value of more than 90%, considering conventional histopathologic examination as the gold standard. The EVM platform that we optimized for evaluation of small tissue fragments fulfills most of these functional requirements, including ease of use, turnaround time, safety, and desired diagnostic capability. However, the preferred size of the platform suggested by the committee is somewhat smaller than the size of the platform that we used in our study. The committee’s specifications related to cost and charges for evaluation per specimen cannot be addressed at this point because our platform is not yet commercially marketed and the billing codes are not yet established.

A few other researchers have reported the use of EVM for the evaluation of CNB specimens with a CFM platform.
Figure 1. Grayscale (A, D, G, and J) and digitally pseudocolored (B, E, H, and K) confocal fluorescence microscopy images of normal breast, kidney, lung, and liver tissue, and their corresponding hematoxylin-eosin–stained tissue sections (C, F, I, and L) (original magnifications ×100 [A through C and G through I] and ×40 [D through F and J through L]).
Figure 2. Grayscale (A, D, G, and J) and digitally pseudocolored (B, E, H, and K) confocal fluorescence microscopy images along with their corresponding hematoxylin-eosin–stained tissue sections (C, F, I, and L) of correctly recognized invasive ductal carcinoma of the breast; renal cell carcinoma, clear cell type; squamous cell carcinoma of the lung; and adenocarcinoma in the liver, respectively (original magnifications ×100 [A through F] and ×40 [G through L]).
Figure 3. Four cases that were not correctly recognized on confocal fluorescence microscopy images. Adenomatous hyperplasia was misdiagnosed as adenocarcinoma (A). Sheets of alveolar macrophages were wrongly diagnosed as squamous carcinoma (D). Two small foci of invasive ductal and invasive lobular carcinoma of the breast were missed on the corresponding confocal fluorescence microscopy images (G and J). The images in the center column represent digitally pseudocolored sections (B, E, H, and K), and the images in the right column show hematoxylin-eosin–stained tissue sections (C, F, I, and L) of the tissue in the left image (original magnifications ×100 [A through I] and ×40 [J through L]).
similar to that used in our study, or the use of platforms based on other types of optical imaging techniques, such as FF-OCT, fluorescence structured illumination microscopy, light sheet microscopy, and MUSE. The earliest report was from Schiffhauer et al, who tested images generated from 49 image-guided CNBs of the breast using confocal scanning laser microscopy with 5% citric acid and glycercin USP to enhance nuclear visibility in the reflectance confocal images. They found that the confocal images were similar to standard histologic sections of the imaged tissue; however, the sensitivity and specificity of the diagnosis from the confocal images were not evaluated. Using a commercially available FF-OCT platform, Krishnamurthy et al evaluated 35 images of fresh specimens from interventional radiology-guided CNBs of various organs, such as the lung, liver, soft tissue, and kidney; that study showed moderate agreement between the diagnosis made with the FF-OCT images and that made with the corresponding H&E-stained tissue sections. However, the FF-OCT images were found to lack the resolution and sharpness needed for accurate distinction of benign tissue, fibrosis, necrosis, and inflammation from malignant tumor areas.

An improved version of the FF-OCT platform has since been built and is currently under investigation. Dobbs et al used an earlier version of the same CFM platform that we used in our study to evaluate 23 CNB specimens obtained from patients with inflammatory breast carcinoma, and they found moderate agreement between the diagnoses made using the CFM images and those made using the corresponding H&E-stained tissue sections. We used an upgraded version of the same CFM platform used by Dobbs et al with additional improvements optimized for evaluating small fragments of tissue. Our ability to place the tissue easily in the tissue holder slot, scan quickly with seamless stitching of the images within a few minutes, and acquire high-resolution images with instant pseudocoloring enabled us to achieve excellent results in the current study. In recent years, promising results have been obtained with EVM platforms built using the principles of fluorescence structured illumination microscopy, light sheet microscopy, and MUSE. Wang et al demonstrated the utility of fluorescence structured illumination microscopy for rapid high-resolution diagnostic imaging of 34 prostate biopsy specimens suitable for point-of-care diagnosis. They achieved a sensitivity of 63% to 88% and specificity of 76% to 89% with 2 pathologists interpreting the images. The same group also recently showed the utility of structured illumination microscopy as a novel imaging technique for rapid, nondestructive histologic assessment of renal neoplasia. They used 65 CNB specimens stained with a dual-component fluorescent stain containing DRAQ5 as the nuclear stain and eosin as the cytoplasmic stain, achieving a sensitivity of 79.2% and specificity of 95.1% with 1 pathologist reading the images. However, the tissue preparation was more elaborate in that study, requiring tissue staining by 2 agents. In addition, the size and portability of the platform suitable for real-time rapid evaluation of CNB specimens were not clear.

Very recently, Glaser et al reported on the utility of light sheet microscopy for slide-free nondestructive pathologic assessment of surgical specimens, including CNB specimens, with the same level of detail as traditional pathologic assessment. These authors studied a single CNB specimen of prostate tissue and demonstrated that with overnight optical clearing, 3-dimensional volumetric assessment of the entire tissue was possible. However, the suitability and potential utility of that platform for rapid bedside tissue evaluation of small fragments need further investigation. Similarly, Fereidouni et al described a MUSE platform that was built to evaluate tissues encountered in surgical pathology practice. The ease of image acquisition after staining various organ tissues with Rhodamine Hoescht, the quality of the images, and the diagnostic capability based on the concordance of the results between 2 pathologists were impressive. The capability of MUSE to aid in rapid real-time evaluation of CNBs needs to be proven in further investigations of this platform.

It should be noted that most of the recent EVM studies were initial feasibility studies, and the promising potential of these platforms needs to be established before they can be transitioned to clinical use. Among the existing reports of using EVM for tissue evaluation of small fragments, our study is perhaps the largest so far, including 118 tissue samples with superb diagnostic performance, as evidenced by a high sensitivity and specificity. Also, the size and portability of the platform and ability to interpret images remotely are added advantages.

In conclusion, we have demonstrated the suitability of a CFM platform for rapid bedside evaluation (on site or remotely) of grayscale and pseudocolored images generated from small fragments of tissues stained with acridine orange. The ease of operation, portability of the platform, and superb diagnostic capability are useful features for transitioning the platform to clinical use. We are currently undertaking tissue integrity studies to establish the suitability of the imaged tissue for ancillary genomic and proteomic investigations. In addition, we have begun training other pathologists to interpret CFM images using training sets, which will allow us to determine the overall sensitivity and specificity of performance based on the interpretation of practicing pathologists. This represents an important step to advance the technology for incorporation into clinical practice.

The authors would like to thank Caliber Imaging and Diagnostic Inc, Rochester, New York, for providing the confocal microscope for the study. We would also like to thank Erica Goodoff, scientific editor, scientific publications, The University of Texas MD Anderson Cancer Center, for helping us edit our manuscript.

References
1. Pincher M, Zawadzki RJ. Review of adaptive optics OCT (AO-OCT): principles and applications for retinal imaging. Biomed Opt Express. 2017;8(5): 2536–2562.
2. Costopoulos C, Brown AJ, Teng Z, et al. Intravascular ultrasound and optical coherence tomography imaging of coronary atherosclerosis. Int J Cardiovasc Imaging. 2016;32(1):189–200.
3. Sturm MB, Wang TD. Emerging optical methods for surveillance of Barrett’s esophagus. Curr. 2015;66(1):1816–1823.
4. Coda S, Thillainayagam AV. State of the art in advanced endoscopic imaging for the detection and evaluation of dysplasia and early cancer of the gastrointestinal tract. Clin Exp Gastroenterol. 2014;7:133–150.
5. Bührig-Meurs V, Laimer M, Rabinovitz HS, et al. Confocal microscopy in skin cancer. Curr Dermatol Rep. 2018;7(2):105–118.
6. Jain M, Narula N, Salamoon B, et al. Full-field optical coherence tomography for the analysis of fresh unstained human lobectomy specimens. J Pathol Inform. 2013;4:26.
7. Jain M, Robinson BD, Salamoon B, et al. Rapid evaluation of fresh ex vivo kidney tissue with full-field optical coherence tomography. J Pathol Inform. 2015; 6:53.
8. Dalmier E, Salamoon D. Full-field optical coherence tomography: a new technology for 3D high-resolution skin imaging. Dermatol. 2012;224(1):84–92.
9. Ragazzi M, Piana S, Longo C, et al. Fluorescence confocal microscopy for pathologists. Mod Pathol. 2014;27(3):460–471.

Confocal Fluorescence Microscopy—Krishnamurthy et al
10. Dobbs JL, Ding H, Benveniste AP, et al. Feasibility of confocal fluorescence microscopy for real-time evaluation of neoplasia in fresh human breast tissue. J Biomed Opt. 2013;18(10):106016.

11. Krishnamurthy S, Cortes A, Lopez M, et al. Ex vivo confocal fluorescence microscopy for rapid evaluation of tissues in surgical pathology practice. Arch Pathol Lab Med. 2018;142(3):396–401.

12. Longo C, Ragazzi M, Gardini S, et al. Ex vivo fluorescence confocal microscopy in conjunction with Mohs micrographic surgery for cutaneous squamous cell carcinoma. J Am Acad Dermatol. 2015;73(2):321–322.

13. Bennassar A, Vilata A, Puig S, Malvehy J. Ex vivo fluorescence confocal microscopy for fast evaluation of tumour margins during Mohs surgery. Br J Dermatol. 2014;170(2):360–365.

14. Wang M, Kimbrell HZ, Sholl AB, et al. High-resolution rapid diagnostic imaging of whole prostate biopsies using video-rate fluorescence structured illumination microscopy. Cancer Res. 2015;75(19):4032–4041.

15. Liu J, Wang M, Tulman D, et al. Nondestructive diagnosis of kidney cancer on 18-gauge core needle renal biopsy using dual-color fluorescence structured illumination microscopy. Urology. 2016;96:195–199.

16. Glaser AK, Reder NP, Chen Y, et al. Light-sheet microscopy for slide-free non-destructive pathology of large clinical specimens. Nat Biomed Eng. 2017;1(7): pii: 0084.

17. Fereidouni F, Harmany ZT, Tian M, et al. Microscopy with ultraviolet surface excitation for rapid slide-free histology. Nat Biomed Eng. 2017;1(12):957.

18. Qorbani A, Fereidouni F, Levenson R, et al. Microscopy with ultraviolet surface excitation (MUSE): a novel approach to real-time inexpensive slide-free dermatopathology. J Cutan Pathol. 2018;45(7):498–503.

19. Li Z, Tonkovich D, Shen R. Impact of touch imprint cytology on imaging-guided core needle biopsies: an experience from a large academic medical center laboratory. Diagn Cytopathol. 2016;44(2):87–90.

20. Kubik MJ, Brobdel A, Goli H, et al. Diagnostic value and accuracy of imprint cytology evaluation during image-guided core needle biopsies: review of our experience at a large academic center. Diagn Cytopathol. 2015;43(10):773–779.

21. Kubik MJ, Mohammadi A, Rosa M. Diagnostic benefits and cost-effectiveness of on-site imprint cytology adequacy evaluation of core needle biopsies of bone lesions. Diagn Cytopathol. 2014;42(6):506–513.

22. Chang YC, Yu CJ, Lee WJ, et al. Imprint cytology improves accuracy of computed tomography-guided percutaneous transbronchial needle biopsy. Eur Respir J. 2008;31(1):54–61.

23. Rekhtman N, Kazi S, Yao J, et al. Depletion of core needle biopsy cellularity and DNA content as a result of vigorous touch preparations. Arch Pathol Lab Med. 2015;139(7):907–912.

24. Tong LC, Rudomina D, Rekhtman N, Lin O. Impact of touch preparations on core needle biopsies. Cancer Cytopathol. 2014;122(11):851–854.

25. Rajadhyaksha M, Menaker G, Flotte T, Dwyer PJ, Gonzalez S. Confocal examination of nonmelanoma cancers in thick skin excisions to potentially guide mohs micrographic surgery without frozen histopathology. J Invest Dermatol. 2001;117(3):1137–1143.

26. Garreau DS, Karen JK, Dusza SW, et al. Sensitivity and specificity for detecting basal cell carcinomas in Mohs excisions with confocal fluorescence microscopy. J Biomed Opt. 2009;14(3):034012.

27. Mathur S, Fitzmaurice M, Reder N, et al. Development of functional requirements for ex vivo applications of in vivo microscopy systems: a proposal from the in vivo microscopy committee of the College of American Pathologists. Mod Pathol. 2018;31:813.

28. Schiiffhauser LM, Borer JN, Bonfiglio TA, et al. Confocal microscopy of unfixed breast needle core biopsies: a comparison to fixed and stained sections. BMC Cancer. 2009;9:265.

29. Krishnamurthy S, Cortes A, Sabir S, et al. Feasibility of using ex vivo full field optical coherence tomography for immediate assessment of tumor cellularity in image guided core needle biopsies. Lab Invest. 2013;95:518A.

30. Dobbs J, Krishnamurthy S, Kyrish M, et al. Confocal fluorescence microscopy for rapid evaluation of invasive tumor cellularity of inflammatory breast carcinoma core needle biopsies. Breast Cancer Res Treat. 2015;149(1):303–310.

---

**CAP19 Abstract Program Submission Deadline Approaching**

Abstract and case study submissions to the College of American Pathologists (CAP) 2019 Abstract Program are now being accepted. Pathologists, laboratory professionals, and researchers in related fields are encouraged to submit original studies for possible poster presentation at the CAP19 meeting.

Submissions will be accepted until 5 p.m. Central Friday, March 8, 2019. Accepted abstracts and case studies will appear on the Archives of Pathology & Laboratory Medicine Web site as a Web-only supplement to the September 2019 issue.

Visit the CAP19 Web site (www.thepathologistsmeeeting.org) or the Archives Web site (www.archivesofpathology.org) for additional abstract program information including a link to the submission site.