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REVIEW ARTICLE

Current landscape and emerging opportunities for using telecytology for rapid on-site assessment in cytopathology

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Abstract

In recent years, cytopathology practices increasingly are considering the adoption of digital modalities to support remote rapid on-site evaluation (ROSE) of fine-needle aspiration biopsies. Currently, various digital options are available, each of which has unique advantages and limitations. This review covers all relevant aspects of telecytology for ROSE, including digital pathology options, operators, validation, quality assurance, reimbursement, and recommendations from professional organizations. The evolving landscape of telecytology for ROSE, including the development of devices for standardized specimen preparation and staining, next-generation digital microscopy techniques, and deep-learning–based artificial intelligence tools as decision-support aids for the interpretation of digital images, also is outlined.

KEYWORDS
artificial intelligence (AI), digital cytopathology, digital microscopy, rapid on-site evaluation (ROSE), telecytology

INTRODUCTION

The field of cytopathology has witnessed revolutionary changes in the last few decades that have enabled the effective use and characterization of cytologic specimens. The field has been significantly advanced by the development of monolayered and cytospin preparations of gynecologic, exfoliative specimens and rinses of fine-needle aspirations (FNAs) collected in liquid fixative and transport medium, as well as various techniques for gathering cells and tissue fragments in liquid medium to create tissue blocks.[1–4] Ancillary techniques that revolutionized standard-of-care surgical pathology practice, including immunohistochemistry and molecular testing, can also be applied to cytologic material, provided the cellular material is sufficient.

Recently, the culmination of technological advances in the fields of engineering and computer science have allowed for the digitization of glass slides with stained tissue samples. The remarkable advancements in digitalization in the field of anatomic pathology led to rapid evolution of the field of digital pathology.[5] Digital pathology involves digitally managing all components of the practice of anatomic pathology, starting from tissue accession and processing; image acquisition of glass slides; and image management, retrieval, review, reporting, storage, and archiving.

Interestingly, both digital images and machine learning–based/deep learning–based artificial intelligence (AI) algorithms were first used for the evaluation of thin preparations of gynecologic specimens and were available to cytopathology practitioners as ancillary aids to evaluate Papanicolaou smears.[6,7] Then, robotic microscopes became available, allowing for the remote navigation of glass slides placed on the microscope and the acquisition of digital images of frozen sections in the field of surgical pathology; however, such advances were

not used in the field of cytopathology.[8] In recent years, tissue scanners that obtain whole-slide images (WSIs) of glass slides have transformed the field of anatomic pathology. Similar to other advances, almost all efforts in digitalizing anatomic pathology practice in the past few years have focused on surgical, but not cytopathologic, specimens: namely, the use of formalin-fixed, paraffin-embedded tissue sections. It is well recognized that the use of WSIs in cytopathology lags behind that in surgical pathology.[9–11]

However, in contrast to the evolution of digital pathology for surgical pathology, cytopathology has been at the forefront of using digital modalities for rapid on-site assessment (ROSE) of cytologic material in the past decade. Community and academic practices are increasingly embracing the use of digital modalities for ROSE in cytopathology.

In this review, the current landscape of telecytology for ROSE in cytopathology is covered. Herein, an overview of the published literature and recommendations of professional organizations regarding the use of telecytology for ROSE are provided. Finally, the emerging field of next-generation digital modalities, devices for standardized specimen preparation and staining, and the potential applications of AI relevant to telecytology practice also are outlined.

NEED FOR TELECYTOLOGY FOR ROSE OF CYTOLOGIC MATERIAL

ROSE of FNA smears and of touch preparations (TPs) from core-needle biopsy allows representative sampling and procurement of diagnostic material, enabling not only accurate categorization and diagnosis but also successful performance of ancillary immunohistochemical and molecular testing.[12–15] Optimal ROSE entails evaluating specimen adequacy, categorizing cytologic material, and determining a preliminary diagnosis of the lesion. Most ROSE procedures use air-dried, Diff-Quik (DQ)-stained FNA smears and TPs to determine specimen adequacy.[16] Several reports attest that ROSE decreases nondiagnostic rates and improves optimal practice of precision medicine.

Whereas core-needle biopsies are increasingly used in current medical practice, FNA remains as a popular modality for image-guided sampling of lesions in standard-of-care clinical practice. The use of FNA is well recognized in the following procedures: ultrasound-guided FNA of the thyroid, endoscopic ultrasound (EUS)–guided FNA of the pancreas, endobronchial ultrasound (EBUS)–guided FNA of the lung and mediastinal lymph nodes, ultrasound-guided FNA for other lymph nodes, and interventional radiology–guided FNA of lesions in deep organs. Therefore, ROSE of FNAs from different organ sites is a common cytopathology procedure.

The types of personnel who perform ROSE vary across the field. Pathology residents, cytopathology fellows, and cytotechnologists routinely perform ROSE with or without the consultation of cytopathologists in some cytopathology practices.[16] It is impractical and unfeasible for a cytopathologist to be physically present at each FNA procedure to evaluate the aspirated specimen in real time or to provide an opinion on cases handled by other members in the team, such as trainees and technologists. However, several practices require cytopathologists to support ROSE of FNAs not only at different locations in the same hospital but also at different satellite centers, which may be distributed far away from the main center.

Although different types of personnel routinely perform ROSE of FNAs, the applicable billing codes for ROSE of FNAs and TPs in United States (i.e., Common Procedural Terminology [CPT] code 88172 [aspirate smear, first pass]; CPT code 88177 [aspirate smear, all subsequent passes]; CPT code 8833 [TP, first pass]; and CPT code 88334 [TP, all subsequent passes]) can be applied only if a cytopathologist performs the immediate FNA assessment when the patient is in the room during the procedure. Therefore, the need for digital modalities to remotely perform ROSE is increasing.

SELECTION OF DIGITAL MODALITY FOR ROSE

The entire cytology team, including faculty and cytotechnologists, should first decide to adopt telecytology for ROSE, with the medical director and laboratory manager making the final decision. After the decision to consider telecytology is made, the active involvement of the administrative leadership and important stakeholders who benefit from ROSE, such as interventional radiologists, oncologists, pulmonologists, gastroenterologists, and surgeons, can be valuable in garnering support for the adoption of telecytology for ROSE.

The next step in the selection process is to evaluate the different ROSE platforms, including their limitations and advantages, and their vendors to determine the most suitable platform and vendor for a given practice. Recently, several different types of telecytology devices for ROSE have emerged because of the increased demand for such devices. Digital cytopathology requires good coordination and communication among a team of experts, including hospital information technologists and laboratory information system personnel, clinical informatic specialists, and the domain expert (i.e., cytopathologists and cytotechnologists). A coordinated effort of the cytology team facilitates the selection and incorporation of a telecytology modality for ROSE in any given practice. Finally, a discussion with the administrative team is warranted to budget for and purchase the device once the team selects a modality.

COMMON DIGITAL MODALITIES FOR ROSE

There are different options for selecting a digital telecytology platform for ROSE, each with its own unique advantages and limitations. Some of the commonly used modalities are summarized below.

Camera-based, static telecytology

A simple telecytology option includes using a camera attached to a microscope and transmitting static images of selected areas of

microscopic slides using email, smartphone apps, or cloud-based secure sites. Alternatively, the on-site FNA operator can also share their computer screen using conferencing software, such as Microsoft Teams, Zoom, or Google Meet, allowing the remote viewer to see the glass slide as it appears under the light microscope.

Although sending static images may be convenient, the ROSE operator has to be skilled in maneuvering the slide on the microscope and capturing adequate representative areas for the remote viewer. Several studies have reported concordance rates of up to 89% when using static images on a computer monitor to provide consultations and primary diagnoses compared with a conventional examination using a light microscrope.[17–22] In fact, Sahin et al. reported an 84.3% concordance rate when viewing static images on WhatsApp.[23]

Camera-based static telecytology for ROSE can be used in low-volume practices and as a backup option for other digital strategies. However, this modality is limited by the size of the selected areas, the necessary skill of the ROSE operator to select adequate and relevant areas, the less-than-optimal image resolution, and the time needed to send the static images to the remote viewer. These limitations make this option less appealing than other telecytology options, particularly for high-volume practices.

Video camera–based, live-streaming telecytology

Another option for telecytology for ROSE is using a high-definition video camera to stream live images directly from a light microscope to a remote viewer. Currently, several video camera–based, live-image streaming telecytology modalities for ROSE are commercially available (e.g., Realtime Telepathology Imaging System [Meyer Instruments, Inc.], ROSE Now-Viewer [Remote Medical Technologies]). The video of the images is streamed over the internet behind an institution's firewall. High-speed internet allows for the streaming of images without notable lag, allowing the on-site driver and the remote viewer to view the entire FNA smear in real time. However, the on-site operator has to be skilled in navigating the slide, similar to the on-site operators who use camera-based telecytology. The specimen identifiers, such as medical record number, patient name and age, and type of specimen, must be communicated over the phone.

The video camera option allows multiple viewers to be connected using the unique internet protocol address created for a single system. Figure 1 shows the setup of a video camera–based, live-streaming telecytology system for ROSE. Generally, the on-site cytotechnologist streams the image on their microscope, either in a dedicated cytology laboratory space adjacent to the site where FNA was performed or in a mobile cart. The cytotechnologist and all remote viewers communicate by phone conferencing to discuss the findings, including specimen adequacy and a preliminary diagnosis. The cytopathologist then communicates the ROSE findings to the radiologist over the phone. There are several reports of successfully using this digital option for ROSE.[24–34]

Hybrid telecytology platforms

Hybrid platforms, including digital microscopes with whole-slide scanners and robotic microscopes with video cameras, allow remote users to navigate the slide and view the images of glass slides in real time. The commercially available robotic microscope with video camera (ROSE Now-Robotic; Remote Medical Technologies) can allow live-dynamic telecytology with robotic microscope control remotely. The digital microscope with whole-slide scanner provides a live view option or a WSI option. One such system, (MOTIC Pro Scan 6; MOTIC Digital Pathology), can scan six slides at a time and upload high-quality WSIs to slide-management servers. Slides can be

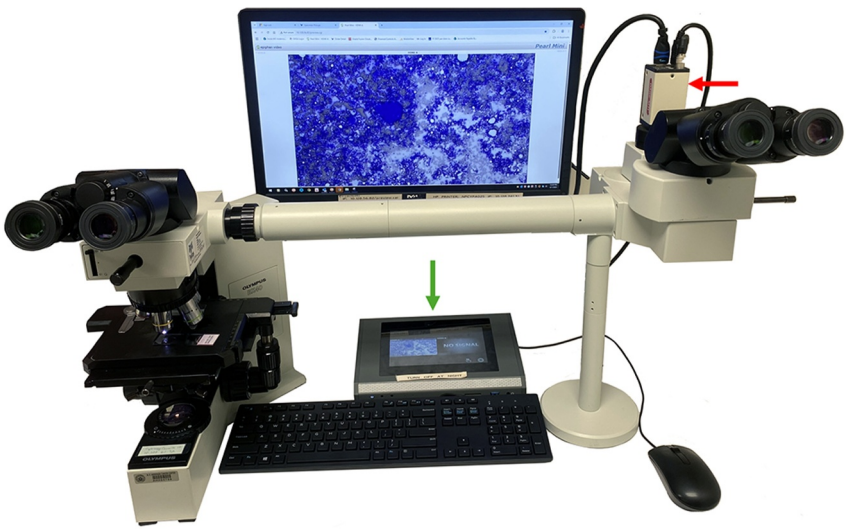

FIGURE 1 Illustration of a video camera–based digital option (Realtime Telepathology Imaging System; Meyer Instruments, Inc.) that can be used for rapid on-site evaluation of fine-needle aspiration biopsies. The system includes a compression–decompression device (green arrow) with its own built-in web server that allows users to view a live signal from a fully automatic, high-definition video camera (red arrow).

scanned at ×20, ×40, and ×80 objective magnification at 0.52, 0.26, and 0.13 μm/pixel resolutions. Another portable hybrid platform (Ocus R Microscope Slide Scanner; Grundium Oy), with live-viewing and WSI options, can be connected to a computer using an institutional internet connection and behind an institution's firewall. The device can be used to view slides in real time with remote navigation or to obtain WSIs, which can be exported in SVS (slide and viewable storage) format or TIFF (tag image file format) format and saved to a dedicated folder by following institutional mandatory rules or can be integrated into the laboratory information system. The device acquires WSIs at 0.48 μm/pixel resolution and at ×20 or ×40 objective magnification. Although the live-view functionality of these hybrid platforms allows for dynamic real-time, seamless viewing, the remote viewer may experience a slight lag in viewing the changes made remotely. WSI scanning provides a better experience than the live-view option, although scanning a WSI takes at least 2–3 minutes per glass slide. The suitability of the live-view or WSI option of hybrid platforms for telecytology and ROSE is currently being investigated. Soriano et al. recently provided data regarding the feasibility and suitability of using the WSI option of a hybrid platform for ROSE of FNABs procured from various body sites.[35] Figure 2 illustrates a hybrid platform with live-viewing and WSI options that can be used for ROSE.

Table 1 summarizes the different digital modalities that can be used for ROSE.

TELECYTOLOGY OPERATORS

The on-site operators of telecytology for ROSE include pathology residents, cytopathology fellows, cytotechnologists, and medical technologists. A survey by the American Society of Cytopathology (ASC) indicated that cytotechnologists are the on-site operators in the majority of practices.[16] The learning curve of on-site operators

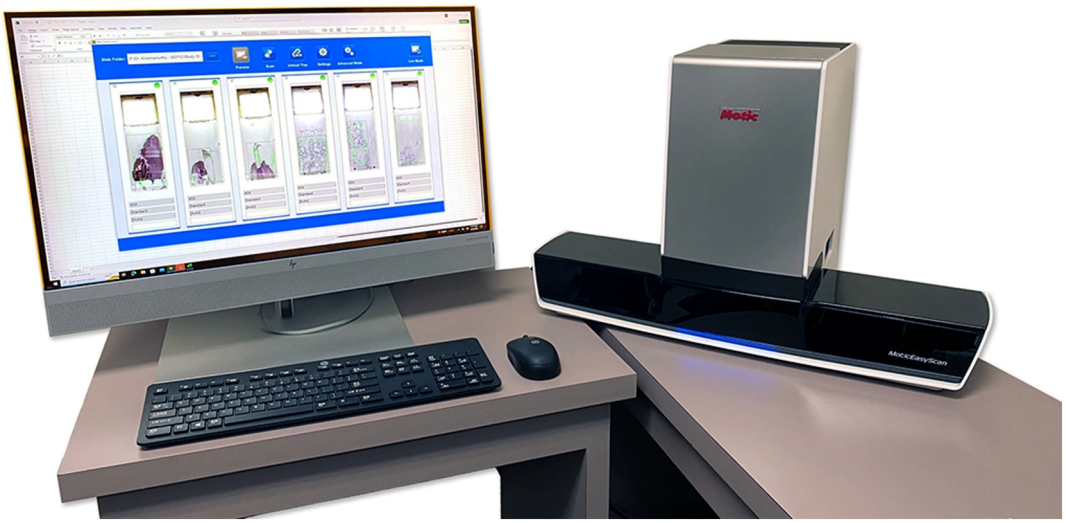

FIGURE 2 Illustration of a hybrid digital pathology option including a digital microscope for live viewing and a scanner for the acquisition of whole-slide images (MOTIC Pro Scan 6; MOTIC Digital Pathology).

TABLE 1 Digital modalities for rapid on-site evaluation of cytopathology specimens.

Digital modality	Cost	Image quality	Time to acquire digital image remotely	Cytology skill of on-site operator
Camera-based	≈$5000	Good	Immediate if computer screen shared Within a few minutes if static images sent	Required
Video camera	≈$12,000–$45,000	Good	Immediate	Required
Robotic microscope with video camera	≈$60,000–$85,000	Good	Immediate with a lag	Not required
Digital microscope and whole-slide scanner	≈$30,000–$55,000			
Live-view option using digital microscope		Good	Immediate with a lag	Not required
Whole-slide imaging option		Good	2–3 minutes per slide	Not required

and the cytopathologists' ability to perform ROSE using digital modalities generally improves with time. In fact, errors by on-site operators are more likely to occur in the first year of adopting telecytology.[36] Furthermore, the ROSE skills of trainees improve during their training, and interpretive errors are more likely to be made by junior cytopathologists early in their practice after finishing fellowship training.[37–39] Camera-based and video camera–based telecytology platforms require and rely on the on-site operators (i.e., the cytotechnologists and trainees) to recognize which findings on the glass slides should be remotely presented to the cytopathologist. In contrast, hybrid devices, including robotic microscopy with video cameras, digital microscopy, and WSI systems, can compensate for on-site operators who may not yet be proficient in navigating the glass slide and making cytomorphologic evaluations. An ASC task force of 13 international groups of cytopathologists and cytologists recently provided recommendations for telecytology of ROSE, including an overview of telecytology platforms, personnel requirements, types of cases to be used for validation, facilities to be included for validation, and acceptable concordance rates.[40] Notably, the recommendations state that, irrespective of the adopted telecytology ROSE modality, it is important to ensure that on-site operators, including trainees, technical staff, and cytopathologists, complete proficiency testing using real-time telecytology cases or prerecorded video clips.[40] Participation in a validation study alone should not be considered a replacement for participating in periodic proficiency training.[40]

VALIDATION OF TELECYTOLOGY FOR ROSE

Telecytology for ROSE of FNA and TPs must be validated before it can be routinely offered as standard-of-care cytology practice. The initial validation study of a telecytology modality essentially evaluates the safety of such a procedure for patient care and its compliance with regulations. Until recently, there were no recommendations for the validation of telecytology for ROSE. Laboratories relied on the College of American Pathologists (CAP)'s published guidelines on validating WSIs of tissue sections for primary diagnosis in surgical pathology, although the cytology specimen preparation and stains, as well as the objectives for telecytology for ROSE, are very different from those for surgical specimens. The CAP guidelines for the validation of WSIs recommend using a minimum of 60 WSIs for each application and achieving a minimum concordance rate of 95% for the diagnosis, with corresponding light-microscopic examination of glass slides after a washout period of 2 weeks.[41]

Telecytology validation should ensure the competency of the personnels involved in ROSE, including cytopathologists, cytotechnologists, and medical technologists; a fully functioning telecytology modality; the availability of digital images of acceptable quality; optimal connectivity for digital review; and most importantly, compliance with patient confidentiality and HIPAA (the Health Insurance Portability and Accountability Act of 1996) rules.[40] These studies should include all locations where ROSE of FNAs and TPs is performed. The number of clinical specimens included in the validation studies can be the same for all sites or can vary based on the procedure volume at the respective sites. Based on available evidence in reported studies, validation of multiple sites can be performed simultaneously or consecutively. Two studies have proposed a staged validation approach of different sites. Monaco and Pantanowitz reported validating more than 60 cases at their main facility, which they identified as their site with the greatest need for ROSE, and subsequently validated additional sites using a smaller number of cases per site.[42] Trabzonlu et al. also reported a staged validation process during which they validated one or a few locations using a few cases, followed by a larger validation using multiple locations.[43]

The cases selected for validation of telecytology for ROSE can include randomly selected or consecutive FNA and TP cases that were retrospectively or prospectively collected. The cases used for validation should be representative of routine clinical practice, including the specimen source of FNAs and TPs and all slides from each FNA pass that were used for immediate assessment. The validation cases should include nondiagnostic and diagnostic cases, with optimal and limited cellularity comprising a good mix of benign and malignant diagnoses.[24] The same slides used for ROSE using light-microscopic examination, which can include DQ-stained slides without a coverslip or hematoxylin and eosin–stained and Papanicolaou-stained smears with a coverslip, should be used for telecytology validation.

Although cytology validation studies have included 60 cases, similar to the CAP requirement for validating WSI modalities for primary diagnosis, the cytology director can select the specific number of cases used for validation and the desired concordance rate.[40] The diagnostic concordance between specimen adequacy and preliminary diagnosis determined on digital images should be compared with the diagnosis made on review of glass slides after a washout period of at least 2 weeks to establish intraobserver concordance. The minimum 95% concordance rate recommended by the CAP guidelines for WSIs of tissue sections for primary diagnosis may not be applicable to telecytology validation studies because of the recognized differences between surgical pathology and cytopathology practice. Some of the telecytology validation studies reported minimum concordance rates of 90% to indicate satisfactory competency for telecytology modalities. When 90% concordance rates were not achieved, repeat validation after a washout period, as well as evaluating the cause of the discordance and making the necessary corrections, has been suggested.[31–33,36,40] Although the recent ASC telecytology validation recommendations do not specifically mention adequate concordance rates, concordance rates of at least 90% for the two objectives of ROSE (i.e., specimen adequacy and preliminary diagnosis) should be considered.[40] Finally, the ASC recommendation acknowledges that a meta-analysis of the telecytology literature to determine the minimum number of cases necessary for telecytology validation has not yet been reported.

QUALITY ASSURANCE AND QUALITY IMPROVEMENT OF TELECYTOLOGY

The integration of telecytology into the laboratory quality-management system is necessary to maintain the high quality of the service.[39] In fact, the CAP accreditation checklist for laboratories includes *telepathology and remote data assessment* and *real-time evaluation of FNA specimens for triaging and preliminary diagnosis*. The quality-management system in cytopathology should include preanalytical factors that can influence the quality of digital images, such as the quality of the smear and staining, the presence of air bubbles, and the competency of technologists and cytopathologists as end users. Federal, state, and local regulations as well as patient confidentiality and HIPAA requirements must be followed. The final cytology report should include the result of immediate assessment, including categorization and documentation of telecytology use, as well as the category III digital pathology add-on codes, which are discussed below. Quality-assurance measures should routinely monitor concordance rates between rapid assessment using telecytology and the final cytology report and should evaluate the cause of discrepant diagnoses and the corrective actions taken to address such discrepancies.[40]

Errors in telecytology commonly occur because of the operators' limited experience. Incorporating continual education and proficiency testing as part of telecytology quality improvement may help to decrease operator errors.[38] Careful monitoring to evaluate the competency of the operators and providing training and feedback and taking timely corrective actions can help ensure that operators are competent. Training and remedial education can incorporate routine cases or short video clips of established cases. Technical issues can be encountered because of problems with the imaging device, problems with video streaming, remote medical technology system failure, network connection problems, password failures, and poor image quality. Coordination with institutional information technology departments to get their support to maintain the hardware/software requirements of the telecytology operations can be critical.

The most common reasons for discordance in adequacy assessment or preliminary diagnosis include not showing the slides with diagnostic material or not showing the representative areas of interest when using camera-based or video camera–based systems. In addition, interpretive errors, internet connectivity issues, and technical artifacts may cause differences between preliminary categorization and the final diagnosis. Although the reported concordance rates of telecytology for ROSE are high, adhering to appropriate quality-assurance measures can help maintain and improve ROSE quality. These quality-assurance efforts can help minimize or avoid incorrect initial categorization, such as nondiagnostic instead of adequate or benign instead of malignant, or can help avoid issues that arise from not sending the aspirated material for ancillary testing, such as not sending a sample for flow cytometry when lymphoma was misdiagnosed as carcinoma or not sending a sample for microbiologic culture study when a possible infection was not recognized during ROSE. Errors should be categorized according to their cause, such as on-site operator, technical, connectivity, or interpretive issues. The corrective action taken should be documented when quality-assurance errors occur. Including the possible issues with telecytology for ROSE in the training material and cytology laboratory procedure manual can make such material good reference material and decrease potential issues with ROSE using telecytology. Discussing the errors and their solutions during quality-assurance conferences is good practice for educating the team routinely in any cytology practice.

REIMBURSEMENT FOR USING TELECYTOLOGY FOR ROSE OF FNAS

This section regarding reimbursement for using telecytology for ROSE of FNAs pertains to cytopathology practices only in the United States. Currently, insurance companies do not provide reimbursement for the technical charges incurred by using telecytology for performing ROSE of FNAs and TPs in the United States. The CAP worked with the American Medical Association procedural terminology editorial panel to release 13 category III add-on digital pathology codes to be added to the appropriate category I code for conventional light-microscopic examination for a defined service.[44] These codes went into effect in January 2023, allowing for providers to report additional service requirements associated with digitizing glass microscope slides to determine a primary diagnosis. Subsequently, 30 additional add-on codes, including four for telecytology for ROSE of FNAs and TPs, went into effect in January 2024.

Although these digital pathology codes have not yet been assigned national pricing from Medicare or other third-party payers, they will allow payers to collect data and determine consistent pricing for add-on services. Each digital pathology add-on category III CPT code has to be reported as a one-to-one unit of service for each pathology service code. Documenting the digital pathology codes pertinent to telecytology within individual practices will be useful when determining the pricing for the codes and for the eventual escalation of the category III to category I codes for realization of the reimbursement for using telecytology. The add-on digital pathology codes pertaining to telecytology are as follows: +0835T, 88172 (digitization of glass microscopic slides for cytopathology evaluation of FNA, immediate cytohistologic study to determine adequacy for diagnosis, first evaluation episode, each site); +0836T, 88177 (immediate cytohistologic study to determine adequacy for diagnosis, each separate additional evaluation episode, same site); +0843T, 88333 (cytologic examination [TP, squash preparation], initial site); +0844T, 88334 (cytologic examination [TP, squash preparation], each additional site); 88172 (aspirate smear, first pass); 88177 (aspirate smear, all subsequent passes); 88333 (TP, first pass); and 88334 (TP, all subsequent passes).

AI–BASED DECISION-SUPPORT AIDS FOR ROSE

In recent years, the development and validation of AI-based decision-support aids for ROSE have received a lot of attention. Few reports have elucidated the potential utility of AI models that can support the

interpretation of EUS-FNA for solid pancreatic lesions and EBUS-FNA for lung lesions. Although some studies used WSIs of DQ-stained smears to develop and validate AI models, others used static images to categorize specimens as adequate or inadequate and benign or malignant.

Lin et al. validated an AI model that was developed using EUS-FNA specimens of solid pancreatic lesions obtained from 51 patients as a potential substitute for manual ROSE.[45] Those authors used 367 static images of DQ-stained smears for training, 100 static images for internal validation, and an additional 693 static images for external validation. The ROSE-AI model achieved accuracy rates of 83.4% for the internal and 88.7% for the external validation data sets, with sensitivity and positive predictive values of 79.1% and 71.75%, respectively, for the internal validation data set and 78.0% and 60.7%, respectively, for the external data set. Fujii et al. recently reported the performance of a ROSE-AI model that was trained using 4059 WSIs of DQ-stained EUS-FNA specimens obtained from 27 patients with pancreatic cancer and nine patients with nonpancreatic cancers.[46] Among their reported techniques, the augmentation of their data using a geometric transformation technique produced the highest diagnosis accuracy rate of 88.2% for the categorization of EUS-FNA specimens as either benign or malignant.

Ai et al. developed an AI model to address the time and personnel needs that may be confounding factors for incorporating ROSE for EBUS specimens.[47] Their AI model was based on deep-learning convolutional neural networks to classify WSIs of EBUS-FNA specimens as either benign or malignant. They used one representative EBUS-FNA slide with representative material from 374 patients for training, 91 patients for internal validation, and an additional 162 patients for testing. The AI model achieved an accuracy rate of 84.57% compared with the 83.3% and 96.90% achieved by two junior cytopathologists who performed ROSE. The ground truth in this study was determined by a senior cytopathologist who later interpreted the slides and generated the official cytology report of the EBUS-FNA specimens. Very recently, Yan et al. developed a ROSE-AI model based on a deep convolutional neural network to categorize EBUS-FNA specimens.[48] The ROSE-AI model was trained using 6357 static images of DQ-stained EBUS-FNA specimens obtained from 721 patients and demonstrated an accuracy rate of 92.9% and 90.2% for the internal and external testing data sets, respectively. In addition, an experienced cytopathologist and the ROSE-AI model were in almost perfect agreement when diagnosing common types of lung cancers, including squamous cell carcinoma, adenocarcinoma, and small cell lung cancer.

The reported literature regarding the potential use of ROSE-AI models in EUS-FNA and EBUS-FNA specimens is promising, and such models are anticipated to be eventually incorporated after rigorous validation and demonstration of robust performance metrics.

NEXT-GENERATION DIGITAL MICROSCOPY FOR ROSE

Optical imaging techniques use light in the visible and adjacent spectra, resulting in the acquisition of tissue images after the interaction of photons with labeled or unlabeled components in tissues.[49] These techniques are essentially optical sectioning microscopy techniques that can obtain digital images directly without the need for the elaborate tissue preparation or staining required for light-microscopic examination. The additional step of digitizing glass slides during light-microscopic examination can be avoided in optical imaging techniques because these techniques are inherently digital. The optical principles behind ex-vivo tissue imaging techniques vary, and some techniques can be used on fresh tissue without applying labeling agents, whereas others require the application of fluorescent dyes to improve contrast for better tissue recognition. Several optical imaging techniques can acquire digitally colorized tissue images resembling images of hematoxylin and eosin–stained tissue.

Most investigations related to ex-vivo optical imaging techniques for the real-time evaluation of fresh tissues have been focused on surgical pathology applications. However, in recent years, some optical imaging techniques, such as fluorescence confocal microscopy, full-field optical coherence tomography, and stimulated Raman spectroscopy techniques, have been explored for potential use in cytology, particularly for ROSE of FNA specimens. Grieve et al. used full-field optical coherence tomography to study 24 EUS–fine-needle biopsy specimens obtained from the pancreas, stomach, and lymph nodes.[50] Although tissue architectural abnormalities could be identified, cellular details could not be appreciated with full-field optical coherence tomography.

Fluorescence confocal microscopy is feasible for evaluating smears and cell pellets and can be used for the acquisition of digitally colorized images in 2–3 minutes, allowing for the accurate categorization and diagnosis of cytologic specimens.[51] Stigliano et al. reported the results of a prospective study of 81 EUS fine-needle biopsies of solid pancreatic lesions; the sensitivity, specificity, and accuracy rates of fluorescence confocal microscopy were 100%, 66.7%, and 97%, respectively.[52] There was 95% correlation with subsequent histologic diagnoses of the pancreatic EUS fine-needle biopsy specimens. Subsequently, Amendoeira et al. compared the interpretations made on 25 EUS fine-needle biopsy specimens of pancreatic lesions with the corresponding histologic WSIs of the specimens by a team of 10 pathologists from the United States, Japan, and Europe.[53] There was substantial agreement among the diagnoses made using the two different modalities.

A prospective clinical study that uses stimulated Raman spectroscopy for EBUS-FNA, including the development of an AI model for decision support in the interpretation of stimulated Raman histology images, is currently ongoing at The University of Texas MD Anderson Cancer Center. Table 2 provides a list of the next-generation, ex-vivo digital microscopy techniques that have

TABLE 2 Next-generation ex-vivo digital microscopy for potential rapid on-site evaluation of cytology specimens.

Ex-vivo optical imaging modality	Use of fluorescent dye	Acquisition of real-time hematoxylin-and-eosin–like digital images
Full-field optical coherence tomography (FF-OCT)	No	No
Fluorescence confocal microscopy (FCM)	Yes	Yes
Stimulated Raman spectroscopy (SRS)	No	Yes

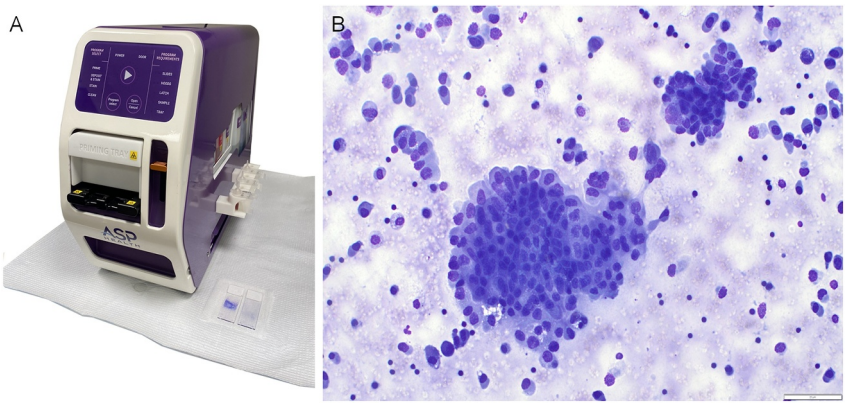

FIGURE 3 Illustration of a device for automated specimen preparation and Diff-Quik staining suitable for telecytology for ROSE (ASP Health). A specimen of pleural fluid prepared and stained with Diff-Quik using (A) the device showing (B) metastatic adenocarcinoma. ROSE indicates rapid on-site evaluation.

potential to be used as telecytology digital options for ROSE. The existing literature suggests that ex-vivo optical digital imaging modalities have a potential role in telecytology for ROSE; however, such use needs further exploration beyond the initial feasibility studies.

AUTOMATED FNA SPECIMEN PREPARATION AND STAINING FOR ROSE

There is lot of interest in standardizing the specimen preparation for ROSE to create high-quality, limited preparations to enable the optimal use of digital modalities for ROSE. Different approaches are currently evolving to create the needed standardization for specimen preparation. One of the commercially available, video camera–based, robotic microscopy systems is equipped with a device (Cell Solutions) that allows monolayer preparations to be rapidly prepared and automatically stained using the Papanicolaou method. This system is currently used in ROSE practice, but there are no published reports regarding the performance of the system. Another such approach is based on the spray technology that takes in a limited amount of material from a vial, either as is or diluted with agents, such as phosphate-buffered saline or methanol, and the device is programmed to stain the specimen using the DQ method and generates a stained slide in 30 seconds. Very recently, Duke et al. reported the results of a prospective study using this automated sample preparation for 72 EBUS-FNA specimens of lymph nodes obtained from 60 patients.[54] To our knowledge, their single-center feasibility study was the first to compare specimen preparation using the device versus standard-of-care protocols and manual DQ staining. Those pathologists evaluated the specimens split for both preparations using metrics, such as nuclear and cytoplasmic quality, the presence of debris and artifacts, staining quality, creation of a monolayer, and ease of adequacy and diagnosis assessment. They reported that 96.8% of the automatically prepared samples were diagnosed the same as their conventionally prepared counterparts. Figure 3 shows the device for automated specimen preparation and DQ staining. These promising automated approaches for specimen preparation and staining for ROSE can aid telecytology practice but need to be substantiated further by multi-institutional studies before considering their potential incorporation into standard-of-care practice.

CONCLUSIONS

Currently, digital modalities are increasingly adopted in cytopathology practices to remotely support ROSE. Various digital options are available when considering telecytology for ROSE. Optimal specimen preparation, including limited but high-quality preparations and the availability of a well trained team of technologists and cytopathologists, with support from information technology and informatics personnel, can contribute significantly toward successful use of telecytology for ROSE. Attempts to standardize specimen preparation and staining and potential incorporation of deep-learning–based AI tools are emerging advancements that may shape the landscape of telecytology for ROSE in cytopathology in the near future.

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CONFLICT OF INTEREST STATEMENT

Savitri Krishnamurthy reports personal/consulting fees from ASP Health outside the submitted work.

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