As optical imaging technologies advance, pathologists will increasingly be at the forefront of the decision-making process at the point of care. The ability to image cancers and precancers in the examination room promises to expand the core competencies of the discipline of pathology into a new domain: in vivo histopathologic diagnosis.

Twenty years ago, radiologists struggled to develop small catheters that assumed tasks previously performed in the operating room, such as taking biopsies of intraabdominal organs and opening up ducts closed by scarring or cancer. Many of these procedures were painful and complicated. These new services required an anesthesiologist, nurse anesthetist, and staff nurses for pain management and monitoring of vital signs. While a radiologist’s patients were once admitted through other clinical and surgical services, some institutions now have interventional radiology units for post-procedural care, such that the kind of monitoring that goes on in a post-operative care unit is available. While a radiologist once worked a single shift, the new interventional radiologist may run two or three shifts per day. In parallel, biopsies and cytologic aspirates performed in the suite require the presence of cytohistopathologists, technicians, and the proper equipment; pathology is now a part of the radiology suite. This new science, which greatly improved clinical care, required a new kind of team and new facilities for implementation.

As “fine needle aspirates” are increasingly performed by cytohistopathologists, they establish outpatient clinics with exam tables in their departments, near to the necessary equipment, for these procedures. The great advantage of this to all parties is the confirmation of a viable specimen during the procedure, which allows the cytopathologist to repeat the assay until a proper sample is obtained. Again, these procedures required new personnel, facilities, and equipment. This is a fairly recent development that provides an example of how the quality of patient care can be improved by connecting patients directly with pathologists.

There are three classes of emerging technologies that will both necessitate and enable the formation of pathologist–clinician teams: (1) quantitative cytology and histology, along with three-dimensional computed tomographic microscopy; (2) confocal microscopy, both ex vivo and in vivo; (3) and optically active molecular-specific contrast agents (Figs. 1–3).

Over 20 years of research have established that ploidy (gross genomic alterations) is a biomarker that is predictive of recurrence and survival for many cancers [1–4]. Our research group and others have found that liquid-based cervical cytology and automated reading of ploidy using a computer-assisted image analysis system have equal performance to HPV testing (using the Virapap®) and Papanicolaou readings performed by gynecologically specialized cytohistopathologists at two large cancer centers [1–5]. Ploidy, in fact, is our best validated surrogate endpoint biomarker. Quantitative histopathology has recently been established as a prognostic and diagnostic tool [6,7]. Both of these technologies rely on quantitative assessment of nuclei and tissue architecture in two dimensions. A newer technology, optical coherence tomographic microscopy, can visualize fixed tissue in three dimensions, allowing ploidy assessment on intact nuclei [8,9]. This capability promises to transform the process of ploidy measurement.

Our research group and others have demonstrated that ex vivo confocal microscopy can reliably discriminate precancer and cancer from normal tissue [9–13]. This technology can also be used with a patient in the clinic. In vivo confocal microscopy using a flexible fiber optic probe allows clinicians to see nuclear size, density, shape, and texture of tissue in the patient. We have begun imaging cervical lesions in vivo and comparing confocal microscopy to clinical and quantitative pathology, with encouraging results [11–13].

Another group of emerging technologies are molecular-specific, optically active contrast agents [14]. A number of new techniques have bound organic fluorescent dyes, metal nanoparticles, and/or semi-conductor nanocrystals to antibodies and/or aptamers in order to image a wide range of biomarkers in cell cultures and excised tissue [15–17]. The further development of this process may herald the age of real molecular diagnosis.

The promise of advanced optical imaging systems is being realized. All emerging technology must be thoroughly studied as it progresses from the laboratory to the clinic [18]. While the biology of these technologies is well understood, the other elements of technical assessment are ongoing. As these technologies develop further, pilot studies and carefully controlled Phase I, II, and III trials with well-defined endpoints are needed to evaluate technical efficacy and clinical effectiveness. Patient and provider acceptance, as well as cost-effectiveness, must be assessed in both low- and high-resource settings.

All of these technologies have potential for application in the developed and the developing world. Quantitative cytology can reduce the cost of the cervical cytologic screens (Papanicolaou
smear) to approximately $3 US. This can make the Pap smears more affordable across the developing world and save resources that are already severely limited. The value of this technology has been demonstrated over the course of a successful project to screen 150,000 women in China [19]. Quantitative histology workstations are emerging that house large image databases of

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Fig. 1. (A) A conventional two-dimensional 4 μm transverse section of normal cervical epithelium stained with hematoxylin and eosin. (B) An ex vivo en face image using a reflectance confocal microscope at a depth of 50 μm of normal cervical epithelium after the placement of acetic acid. Biopsies are ~200 μm in thickness. (C) An in vivo en face image using a reflectance confocal microscope and fiber optic probe at a depth of 50 μm of normal cervical epithelium after the placement of acetic acid. The field of view is 180 × 170 μm. (D) A conventional two-dimensional 4 μm transverse section of high-grade (cervical intraepithelial neoplasia grade 3 (CIN3)) cervical epithelium stained with hematoxylin and eosin. (E) An ex vivo en face image using a reflectance confocal microscope at a depth of 50 μm of high-grade cervical epithelium after the placement of acetic acid. Biopsies are ~200 μm in thickness. (F) An in vivo en face image using a reflectance confocal microscope and fiber optic probe at a depth of 50 μm of high-grade cervical epithelium after the placement of 6% acetic acid. The field of view is 180 × 170 μm [20]. (G) A scatter-plot contrasts the measurement of the nuclear to cytoplasmic ratio and the average nuclear area as measured with a confocal microscope using ex vivo biopsies of normal, low-grade, and high-grade lesions confirmed with histopathology [17].

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Fig. 2. (A) A conventional two-dimensional 4 μm transverse section of normal cervical epithelium stained with hematoxylin and eosin. (B) A conventional two-dimensional 4 μm transverse section of normal cervical epithelium stained with the Feulgen-thionine stain for morphometric analysis. (C) A conventional two-dimensional 4 μm transverse section of normal cervical epithelium stained with hematoxylin and eosin showing Voronoi tessellation of tissue architecture. (D) A three-dimensional reconstructed image of normal cervical epithelium as imaged with an Optical Computed-Tomographic Microscope. (E) A conventional two-dimensional 4 μm transverse section of high-grade (in this case carcinoma in situ) cervical epithelium stained with hematoxylin and eosin. (F) A conventional two-dimensional 4 μm section transverse of high-grade cervical epithelium stained with the Feulgen-thionine stain for morphometric analysis. (G) A conventional two-dimensional 4 μm transverse section of high-grade cervical epithelium stained with hematoxylin and eosin showing Voronoi tessellation of tissue architecture. (H) A three-dimensional reconstructed image of high-grade cervical epithelium as imaged with an Optical Computed-Tomographic Microscope. (I) Chromatin texture score from an algorithm using nuclear texture measurements and triply confirmed histopathologic diagnosis.
tissue samples and can display a gallery of with diagnoses from actual cases that have been read by experienced pathologists that are similar to the case under consideration. These systems could aid histopathologists in low-resource settings to read the cytology and histology from large screening and diagnostic programs without the need for years of training and confirmation by very senior analysts. In high-resource settings, quantitative cytology can be used to verify a human reading, and quantitative histology could prognosticate the course of suspicious tissue so clinical physicians can better guide future screening and interventions.

Confocal microscopes could be used on the desktop to view ex vivo biopsies to aid in real-time diagnosis. In the developing world, confocal microscopy could allow pathologists to image a specimen without having to fix and stain it. Perhaps only those specimens that are suspicious for invasive cancer would require fixing and staining. In the developed world, this technology could allow us to use expensive frozen section pathology more selectively, reducing costs. If in vivo confocal instruments can be produced cost-effectively, it would be an optimal tool for screening and diagnosis in the developing world. In high-resource settings, in vivo confocal microscopy could be used to better target tissue areas that require biopsy or to evaluate margins in the operating room.

Over the next decade, optically active contrast agents could be used in low-resource settings on the desktop to identify important biomarkers on excised tissue in order to save the cost of immunohistochemical staining. In the developed world, contrast agents shown to be safe for use in patients could be applied topically for (1) in vivo molecular diagnosis, (2) selection of molecularly targeted therapeutics, and (3) monitoring the efficacy of new therapies.

These technologies all provide opportunities to bring pathologists into the clinic to give patients accurate real-time results, targeted biopsy and treatment, and the potential to create cost-effective health programs. As pathologists become part of the clinical team, new billing codes will need to capture their involvement. Point-of-care teams of pathologists and clinicians will lead the paradigm shift to improve global health care.

Conflict of interest statement
RR-K has an ownership stake in Remicalm, Inc which is a commercializing technology related to that described in this paper. All other authors declare that they have no conflict of interest.

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