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Original contribution

Spatial resolution requirements for acquisition of the virtual screening slide for digital whole-specimen breast histopathology☆

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Summary We examined the effect of lateral spatial resolution and reader specialty on the accuracy of detection of breast cancer. The motivation for this pilot study was the need to acquire and display very large data sets in whole-specimen 3D digital breast histopathology imaging. The ultimate goal is to determine the minimum resolution adequate for detection of malignancy. Twenty-three histologic slides were selected from breast pathology cases and digitized at 2 sampling distances (3.2 and 1.9 μm pixels). Images were viewed by 14 pathologists, of whom 5 had breast pathology as their primary specialty. The readers assessed the likelihood of malignancy on a 5-point Likert scale, and provided a provisional diagnosis. For the detection task, sensitivity, specificity, overall accuracy of detection, and area under the receiver-operator curve were calculated. An overall diagnostic score, and scores grouped by malignancy type, were also computed. Outcome measures were examined for significant resolution and specialty effects. Increasing the lateral resolution significantly improved accuracy in diagnosis (P = .004) but no effect was found for detection. Breast specialists achieved significantly higher scores for all outcome measures except specificity. Differences in performance between the 2 groups of readers tended to be greater for the diagnostic task compared to detection, especially at the higher resolution. However, specimen coverage may also be a significant factor. Factors related to the readers may have also affected performance in this study. Based on these results, a more comprehensive study should examine pixel sizes between 0.7 and 1.9 μm.

1. Introduction

Economic and staffing constraints make the use of telepathology for diagnosis and intraoperative consultation a necessity in many centers. Telepathology has been a primary motivator for the development of digital pathology

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imaging systems. Various approaches to telepathology provide different degrees of coverage, meaning the fraction of tissue that is digitized and viewed. In static telepathology, selected "snapshots" of slides are electronically sent for consultation, and in the dynamic approach, the consulting pathologist navigates the slide on a remote microscope, still viewing one field-of-view at a time [1]. Whole-slide imaging systems that can digitize an entire slide into a high-resolution, composite image or "virtual slide" are replacing the static, as well as dynamic and hybrid approaches [1-6]. Coverage is usually the least in static pathology and diagnostic accuracy (concordance with glass-slide diagnosis) can vary widely (76%-100%) depending in part on how the images are selected [1,7-9]. On the other hand, the virtual slide yields a more reliable diagnosis [1,6,10,11].

Digital pathology imaging makes it possible to view and navigate over large volumes of data, which are typically captured from conventional histology slides. To further enhance specimen coverage, we are developing a system for 3-dimensional (3D) breast histopathology imaging of whole specimens using large slides instead. The specimen is prepared into whole-mount serial sections (about 150, 70 × 70 mm sections from an average specimen) as it is maintained in as close as possible to the in vivo conformation [12]. The imaging hardware consists of a modified transmission microscope, with a camera and large-area translation stage [13]. Compared to digital imaging of conventional, small-format histology, the 3D, whole-specimen approach offers far greater coverage of the specimen. Increased coverage is desirable because undersampling is associated with failure to detect malignancy [14,15]. This, in turn, may be associated with underestimating total tumor area, missing a close or involved margin that requires secondary treatment, or failing to correctly identify disease as multifocal or unifocal.

Increasing either coverage or the lateral digitizing resolution increases the amount of image data that must be processed, stored, and displayed. The best resolution available at a diagnostic magnification (20×) is 0.7 μm.[1] To image the entire specimen in the 3D approach at this resolution would produce hundreds of trillions of bytes of image data. In conventional small-format histopathology, work has been done to reduce the amount of image data using sophisticated image compression and other computational methods [16,17].

Breast cancer is commonly initially detected with screening mammography. Suspicious lesions are subsequently characterized by a "diagnostic" imaging workup. We are investigating a similar approach for histopathology to manage the enormous amount of image data. Our approach is to digitize at a reduced sampling rate (number of samples/mm), but one that still permits *detection* of abnormalities in a 3D presentation of the entire tissue sample. Then, for definitive *diagnosis*, suspicious areas can be revisited, if necessary, by automatic stage repositioning for viewing at a higher, diagnostic magnification. This strategy, which separates the processes of screening for abnormalities and diagnosis, is based on the assumption that abnormalities can be detected by using architecture-based patterns alone with limited cellular or nuclear detail, and that a level of resolution that is subdiagnostic is adequate to capture such features. Unlike commercially available virtual slide processors that can digitize conventional size slides at a diagnostic resolution, the "virtual screening slide" is, at present, a research tool that is optimized for screening serial whole-mount sections using a lower resolution.

We examine the effect of lateral resolution while considering the effect of pathologist specialty by using 2 categories of readers: those whose primary specialty is breast, and those who specialize in other sites. Concentration of expertise and the effect of volume have been studied in detail for other specialties, and have been associated with improved outcome after liver and kidney transplantation, for example [18]. For breast, there is some evidence for improved accuracy with specialization [19]. However, this association has not been extensively studied and most hospitals continue to operate with generalists.

The lateral resolution available from the digital image is determined by 2 major factors: the optical resolution of the microscope and the sampling performed during the digitization process. The optical resolution depends on the objective NA and the wavelength of light. The limiting factor for resolution in a digital image is usually the sampling performed in digitization, which is determined by the camera pixel size (pixelation).

The central question in this approach, which this study begins to explore, is the choice of (optimal) pixelation—ie, what is the coarsest pixelation that achieves detection sensitivity equivalent to that of conventional histopathologic evaluation under a microscope? In this pilot study, we test for differences on the rate of detection as well as diagnosis due to lateral resolution and reader specialty effects, using 2 categories for each, and using standard-sized slides.

2. Methods

The study was performed using readers and cases from Sunnybrook and Women's College Health Sciences Centre.[2] A retrospective set of 23 breast cases from 2002 to 2004 was collected and 14 pathologists performed readings of the case set from February 2005 to May 2005.

[1] This assumes that a plan achromat objective is used (numerical aperture [NA] = 0.40) and is calculated by using the Rayleigh criterion for the minimum distance between 2 adjacent dots that appear distinct:

$d = \frac{0.61\lambda}{\mathrm{NA}}$, where λ is an average wavelength of light.

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