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Technical Note

Standardization of whole slide image morphologic assessment with definition of a new application: Digital slide dynamic morphometry

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Abstract

Background: In histopathology, the quantitative assessment of various morphologic features is based on methods originally conceived on specific areas observed through the microscope used. Failure to reproduce the same reference field of view using a different microscope will change the score assessed. Visualization of a digital slide on a screen through a dedicated viewer allows selection of the magnification. However, the field of view is rectangular, unlike the circular field of optical microscopy. In addition, the size of the selected area is not evident, and must be calculated. **Materials and Methods:** A digital slide morphometric system was conceived to reproduce the various methods published for assessing tumor budding in colorectal cancer. Eighteen international experts in colorectal cancer were invited to participate in a web-based study by assessing tumor budding with five different methods in 100 digital slides. **Results:** The specific areas to be tested by each method were marked by colored circles. The areas were grouped in a target-like pattern and then saved as an .xml file. When a digital slide was opened, the .xml file was imported in order to perform the measurements. Since the morphometric tool is composed of layers that can be freely moved on top of the digital slide, the technique was named digital slide dynamic morphometry. Twelve investigators completed the task, the majority of them performing the multiple evaluations of each of the cases in less than 12 minutes. **Conclusions:** Digital slide dynamic morphometry has various potential applications and might be a useful tool for the assessment of histologic parameters originally conceived for optical microscopy that need to be quantified.

Key words: Assessment, digital slide, morphometry, standardization

Video 1 available on www.jpathinformatics.org

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INTRODUCTION

The use of whole slide digital imaging has been limited essentially to frozen intraoperative diagnosis or second opinion consultations. More recently, novel applications have included the evaluation of interobserver diagnostic variability[1] and computer-assisted morphometric quantification of prognostic factors.[2]

In routine histopathology, the quantitative assessment of morphologic features has been developed using fields of view observed with optical microscopes and expressed as areas in mm^2 or magnifications (high-power fields). In addition to using different microscopes and objectives (i.e., magnifications) that yield different areas of observation, investigators may use different cut-offs and approaches, such as subjective evaluation versus objective quantification. Furthermore, an assessment may be performed throughout the whole lesion on multiple slides, or on the most representative slide containing the "hot spot," and/or in multiple randomly selected areas. An example of variation in the selected field areas are the reported mean and standard deviation for the number of colonic mucosal mast cells in a control population, ranging from 13.3 ± 3.5[3] to 37.3 ± 6.0 per high power field.[4] Finally, the cut-off values can be chosen arbitrarily, or based on outcome analysis, or on inter-/intraobserver analysis. An example of variation in cut-off values is the number of eosinophils per high-power field (based on peak count) used to establish a diagnosis of eosinophilic esophagitis. This number has been set at 15 in 10 studies, 20 in 8 studies, 24 in 2 studies, and 30 in 1 study.[5]

In oncologic pathology, the mitotic count is an important prognostic factor, and in breast cancer, is also an essential component of histological grade. However, the count may vary up to 250% because of variation in the area of the high-power fields of different microscopes, and also due to the different methods used for counting mitotic figures and recording results.[6] Although the College of American Pathologists Invasive Breast Cancer Protocol provides a table for adjusting the raw number of mitoses according to the size of the field of the microscope used (either by diameter or area in mm^2)[7] mitotic count cut-offs are subject to important sampling variation, and prognostic or predictive cut-offs have not been well studied.[8]

For gastrointestinal stromal tumors, the need for standardizing the mitotic count according to the surface area examined (based on the size of high power fields) has already been underlined: once more, the problem is that there are no agreed upon definitions.[9]

Tumor budding is a dedifferentiation process occurring at the invading edge of colorectal cancer and several epithelial malignancies. It appears as clusters of undifferentiated cells detaching from the main tumor. Several studies have shown that tumor budding is independently associated with lymph node and distant metastasis as well as shorter disease-free and overall survival, in stages I to III colorectal cancer. However, the assessment of tumor budding is not standardized, and therefore, the clinical impact remains limited.[10]

Several original methods have been proposed for assessing tumor budding in colorectal cancer and can be grouped as follows: methods based on a subjective impression of the overall tumor;[11,12] methods based on counting with a cut-off in the field with the most tumor budding;[13,14] methods developed with counting in different areas.[15]

Furthermore, while some authors report that budding clusters are easily identifiable on hematoxylin and eosin (H and E) stained sections,[14,15] others use immunohistochemistry to better visualize this feature.[16] Such differences in methodology make it impossible to compare data from different studies.

Although digital slide viewers allow the selection of magnification, the field of view (computer screen) is rectangular and therefore differs from the circular fields of view of an optical microscope. Furthermore, the size of the selected area is not immediately evident, and must be evaluated using the draw functions or the scale/axes grid.

However, we opined that whole slide digital imaging may allow to reproduce the various methods efficiently, to compare them, and to evaluate interobserver reproducibility. This paper details a new strategy, *digital slide dynamic morphometry*, that allows precise reproduction of one or multiple methods originally determined for optical microscopy. This system was tested in the frame of an interobserver study assessing the reproducibility of a number of tumor budding scoring methods applied to 50 colorectal cancer cases.

MATERIALS AND METHODS

High-resolution, whole slide images were acquired from 50 H and E slides and corresponding AE1-3 stained cytokeratin sections of 50 stages I-III colorectal cancer cases using a ScanScope CS microscopic scanner (Aperio Technologies, Vista, CA, USA). The 100 histological and immunostained sections were scanned at a magnification of 40×.

Such magnification (40× corresponding to a resolution of 0.25 μm) was adopted to allow zooming without loss of details necessary for identifying single tumor buds. The digitized slides were uploaded to a study website (http://course.path.mgh.harvard.edu/budding_project/) as tiled TIFF 6 files, compressed in JPEG 2000 at quality factor of 70, for online viewing through a digital microscope interface (Aperio ImageScope).

The adoption of jpeg2000 images allowed also a high quality preservation of cellular details.

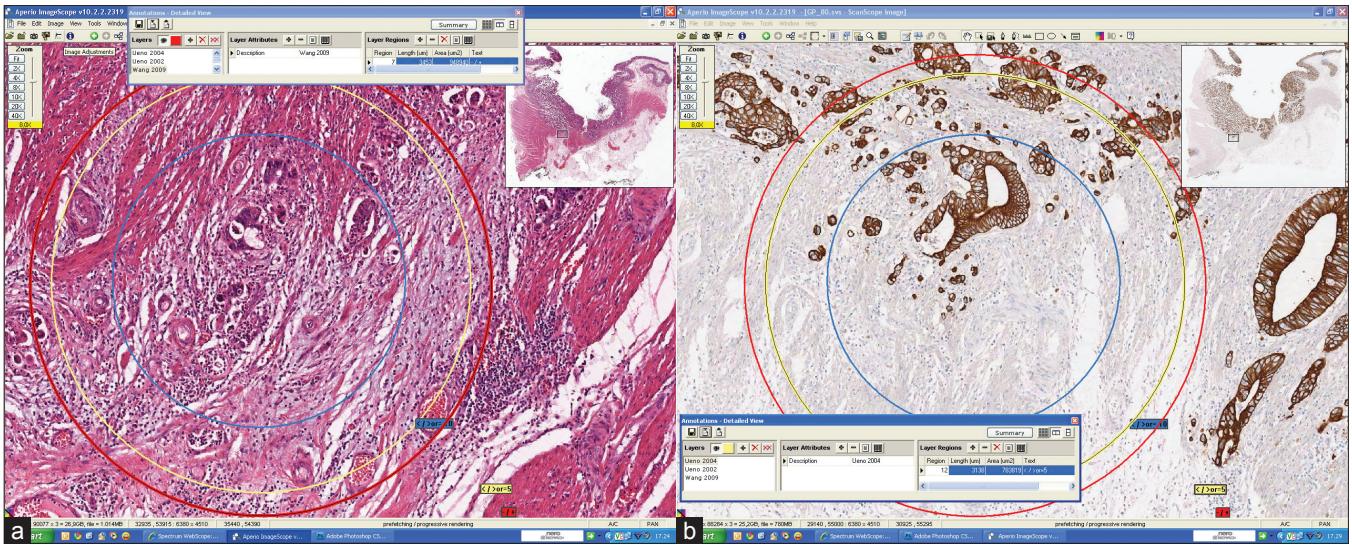

Figure 1: The advancing edge of a colorectal cancer with tumor budding, stained with H and E (a) and by AE1-3 cytokeratin (b). From the Image Scope viewing window, the following elements are selected: Zoom toolbar indicating the magnification; Thumbnail Window showing which part of the entire image is evaluated; Status Bar showing the target coordinates (X and Y). The Annotation panel also is selected, showing the multiple (three) annotation layers, saved in different colors and organized with descriptions (author, year of publication; size area, and cut-off values; the same cut-off value is also highlighted with the corresponding color in the lower right of the circles)

It was possible to apply methods based on subjective evaluation on the whole tumor section, just by navigating into the virtual slide.

An original assessment system was conceived for reproducing the methods based on tumor budding enumeration using cut-off values in the field of maximum budding and also for reproducing the methods proposed for counting budding in different tumor areas [Video 1].

The "draw annotations" function of the Aperio ImageScope viewer was used to draw multiple circles, each reproducing the area for a given method. Finally, the tumoral region with maximum tumor budding ("hot spot") could be recorded thanks to the status bar that provides the *X* and *Y* coordinates of each pixel indicated by the pointer as it moves over the virtual slide. After the center of the target was indicated with the pointer, the coordinates were recorded.

A tutorial video explaining how to import the tool file and use it on the digital slide was prepared with screen video capture software (TechSmith Camtasia Studio 6, Okemos, MI, USA). The video was uploaded into the study website as a windows media file (.wmv).

Eighteen colorectal cancer experts including pathologists, surgeons, and researchers were invited to participate. During the initial contact, the invitees were given basic background information on the aims, methods, and objectives of the study.

The participants who agreed received a folder containing (1) an Excel file to record the tumor budding assessment; and (2) an .xml file containing three colored circles ("targets"), each representing a method to be imported and displayed onto each slide. Finally, the investigators where asked to record the time needed to perform the entire assessment, categorized as <10 hours, 10--15 hours, 20-25 hours, and >25 hours.

RESULTS

Each method based on counting tumor budding within a specific area was reproduced as a colored circle corresponding to a graphic overlay with all the pertinent information: size, cut-off value, author, and year of publication. The various circles were grouped in a target-like arrangement and then exported and saved as an .xml file [Figures 1a-b and Video 1]. When each digital slide was opened, the file containing the target-like area was imported in order to perform the assessment.

Once the target-like area appeared, the field of view could be adjusted with the "zoom toolbar" of ImageScope until the target fit the monitor. Since the area was fixed, magnification was not important in reproducing a method.

Two keyboard keys were used to move the target around the slide. It was possible to move all the circles together while holding the Shift+Control keys or, after having selected one layer, to move a circle separately from the others while holding the Shift key. Thus, the various layers were not merged together or burned into the image, allowing a fully dynamic assessment.

The circles grouped within the target shape showed the variation in field area as viewed by the different investigators in their studies [Figure 1a-b: blue vs. yellow vs. red].

Of the 18 invited investigators, 12 completed the task. Four investigators preferred to perform the assessments as pairs, and so a total of 10 files were submitted. The reasons for the failure of the remaining six investigators to participate included technical problems for two (viewing the tutorial video and opening the digital slides, respectively), and a missed deadline (four).

Six of 10 assessments took less than 10 hours for both H and E stained slides and corresponding cytokeratin immunostains. For two investigators, the assessment of H and E stained slides took longer than for the immunostains (15-20 hours vs. 10-15 hours and <10 hours); for one investigator it was the opposite (15-20 hours vs. 20-25). For the remaining observer, the assessment took more than 25 hours for both stains. Thus, the majority of the individual assessments took less than 10 hours for each 50-case set, i.e., less than 12 minutes per case, keeping in mind that 5 different assessment methods were evaluated for each case.

DISCUSSION

This advanced assessment system allows simultaneous application of different methods originally conceived for optical microscopy by using customized tools prepared specifically to reproduce those methods digitally.

The methods are converted into a morphometric tool, a file to be imported into the digital slide for quantitative assessment.

The graphic overlay layers that compose the morphometric tool can be moved freely over the digital slide, and can be kept separate or grouped. For this reason, the system was named *digital slide dynamic morphometry*. This method has various potential applications.

It may be a useful tool for histopathological assessment of parameters that need to be quantified in a whole slide image (such as the estimation of tumor diameters and the measurement of cancer invasion) as well as within a given area (for example the quantification of specific cells or the mitoses counting) as illustrated in Figure 2.

It also may be a tool for quality control of grading protocols, whose results influence prognosis and therapeutic decisions. Furthermore, the proposed methodology appears to be readily applicable after review of a tutorial (Video 1, Supplemental Digital Content). Finally, the duration of the analyses, less than 12 minutes per case, applying five different assessment methods, that is an average read-time of 2 minutes per method, appears to indicate that the method is also time efficient (the median read-time per slide using a method of budding quantification was 1.3 minutes and 1.7 minutes for two pathologists).[15]

In addition, considering that most of the digital slide viewers allow to draw annotations, the assessing system can be widely used in digital microscopy.

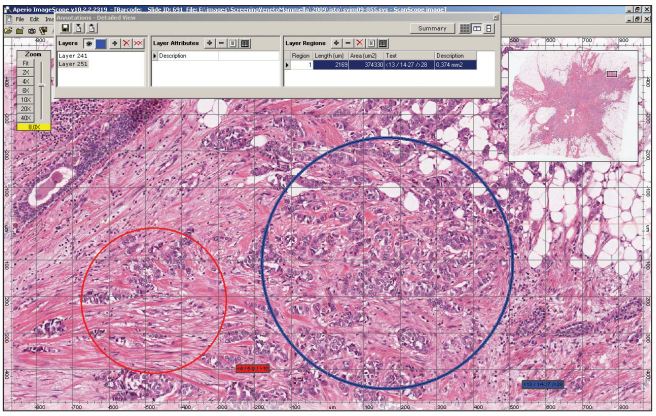

Figure 2: Invasive ductal carcinoma of the breast stained with hematoxylin and eosin: mitotic count standardization. From the ImageScope viewing window, the following elements are selected: Zoom toolbar indicating the magnification; Thumbnail window showing which part of the entire tumor is under evaluation; Scale Axes/Grid allowing mitotic counts mapping and localization. The Annotation panel also is selected showing the multiple (two) annotation layers, saved in different colors, and organized with descriptions (size area and score categories used for tumor grading; the same cut-off values are also reminded, highlighted with the corresponding color, in the lower right of the circles). The red circle (0.125 mm^2) and the blue one (0.374 mm^2), highlighted, correspond to the greatest variation in size of high power fields as viewed with different microscopes (areas extracted from reference[7])

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