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18 | Real-time Reflectance Confocal Microscopy of Two-dimensional Images and Three-dimensional Image Stacks for Detection of Cervical Precancer

ABSTRACT

Confocal microscopy can provide real-time, 2-D and 3-D images of the cellular morphology and tissue architecture features that pathologists use to detect precancerous lesions without the need for tissue removal, sectioning, and staining. The utility of 3-D confocal image stacks of epithelial tissue for detecting dysplasia has not yet been explored. We aim to extract morphometry and tissue architecture information from 2-D confocal reflectance images and 3-D image stacks from fresh, unstained cervical biopsies and compare their potential for detecting dysplasia. Nine biopsies are obtained from eight patients; confocal images are acquired pre- and postacetic acid at multiple epithelial depths in 1.5 mum-intervals. Postacetic acid images are processed to segment cell nuclei; after segmentation, 2-D images taken at 50 mum below the tissue surface, and the entire 3-D image stacks are processed to extract morphological and architectural features. Data are analyzed to determine which features gave the best separation between normal and high-grade cervical precancer. Most significant differences are obtained from parameters extracted from the 3-D image stacks. However, in all cases where the 2-D features were multiplicatively scaled by the depth of acquisition divided by the epithelial thickness or scaled by the scattering coefficient, the significance level is equal to or greater than the comparable feature extracted from the 3-D image stacks. A linear discriminant function previously developed to separate 19 samples of normal tissue and high-grade cervical precancer based on the nuclear-to-cytoplasm (N/C) ratio and epithelial scattering coefficient is prospectively applied to the nine biopsies examined to determine the accuracy with which it could separate normal tissue from cervical intra epithelial neoplasia (CIN) 23. For the entire data set of 28 biopsies, a sensitivity and specificity of 100% is produced using this discriminant function; the scattering coefficient provides more discriminative capacity than the N/C ratio. The success of the scaled 2-D image features has important implications for using confocal microscopy to detect precancer in the clinic. Acquisition of the epithelial thickness or scattering coefficient requires less time than 3-D image sets and little additional effort is required to gain the added information compared to 2-D images alone.