Multimodal confocal mosaics enable high sensitivity and specificity in screening of in situ squamous cell carcinoma

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ABSTRACT
Screening cancer in excision margins with confocal microscopy may potentially save time and cost over the gold standard histopathology (H and E). However, diagnostic accuracy requires sufficient contrast and resolution to reveal pathological traits in a growing set of tumor types. Reflectance mode images structural details due to microscopic refractive index variation. Nuclear contrast with acridine orange fluorescence provides enhanced diagnostic value, but fails for in situ squamous cell carcinoma (SCC), where the cytoplasm is important to visualize. Combination of three modes [eosin (Eo) fluorescence, reflectance (R) and acridine orange (AO) fluorescence] enable imaging of cytoplasm, collagen and nuclei respectively. Toward rapid intra-operative pathological margin assessment to guide staged cancer excisions, multimodal confocal mosaics can image wide surgical margins (~1cm) with sub-cellular resolution and mimic the appearance of conventional H and E. Absorption contrast is achieved by alternating the excitation wavelength: 488nm (AO fluorescence) and 532nm (Eo fluorescence). Superposition and false-coloring of these modes mimics H and E, enabling detection of the carcinoma in situ in the epidermal layer. The sum mosaic Eo+R is false-colored pink to mimic eosins’ appearance in H and E, while the AO mosaic is false-colored purple to mimic hematoxylin’s appearance in H and E. In this study, mosaics of 10 Mohs surgical excisions containing SCC in situ and 5 containing only normal tissue were subdivided for digital presentation equivalent to 4X histology. Of the total 16 SCC in situ multimodal mosaics and 16 normal cases presented, two reviewers made 1 and 2 (respectively) type-2 errors (false positives) but otherwise scored perfectly when using the confocal images to screen for the presence of SCC in situ as compared to the gold standard histopathology. Limitations to precisely mimic H and E included occasional elastin staining by AO. These results suggest that confocal mosaics may effectively guide staged SCC excisions in skin and other tissues.