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HEMATOXYLIN COUNTERSTAIN TO SIMPLIFY WHOLE SLIDE SCANNING OF IMMUNOFLUORESCENCE STAINS

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Introduction/ Background

Whole slide scanning of immunofluorescence (IF) stained slides is a well-recognized need, because fluorescence signal fading makes slides non-archivable. Technically scanning of IF has remained difficult, because tissue finding and autofocus operations are slow and prone to errors under epifluorescence illumination.

Aims

To simplify and to make IF scans more reliable, we started to look for counterstains that are compatible with IF and that can be scanned under brightfield illumination first.

Methods

Of the many alternative counter stains tested, light hematoxylin (H) proved the best counterstain for IF (with or without DAPI). Hematoxylin staining allowed the scanner (OIT Turboscanner) to perform rapid and accurate tissue finding and to define autofocus points, yet it did not increase background autofluorescence or decrease true fluorescence signal during subsequent scanning of IF under epifluorescence illumination. By using Cy2 (green) and Cy3 (orange) as secondary antibody fluorochromes (Jackson Immunotech), we were able to use standard xylene-based DPX mounting medium without observing significant fluorescence signal fading.

Results

As a result, IF scans of H counterstained slides could not be distinguished from those stained without hematoxylin counterstaining. As a bonus, the two scans (IF and H) could be viewed on the viewer screen side-by-side, or by gradually blending the two layers with each other. Both viewing modes were found useful in diagnostic dermatopathology when analyzing e.g. blistering skin lesions. A brightfield-IF scanning combination was also found useful in double immunostains, where cellular co-localization makes use of two precipitating (chromogenic) markers unreliable. The peroxidase IHC-hematoxylin-IF stain was exemplified with Ki-67 and pan-cytokeratin in breast cancer samples. The brightfield WSI of Ki-67 with H counterstain is an easily evaluable chromogenic DAB stain, but the image analysis software (ImmunoRatio2, embedded in the WSI viewer) utilizes the hidden pan-cytokeratin IF to create a cancer mask to exclude counting of non-epithelial stromal and lymphoid cells in the Ki-67 labeling index counting. Together these results demonstrate that a minor modification in the staining protocol (using hematoxylin instead of or in parallel to DAPI as counterstain) provides a significant help for whole slide scanning of immunofluorescence stains. Some scanners may need to be re-programmed to allow scanning of brightfield and epifluorescence automatically in sequence.