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Quantification of IHC Using an Inverted Confocal Microscope and NIS-Elements Program-Killinger Lab 2024

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol to quantify DAB stained sections using NIS-Elements.

Troubleshooting

1 Capture brightfield images with an inverted confocal microscope with a 20X objective (Nikon A1R).

2 Conduct annotation of each tissue section within a bounding box of 2000×2000 pixels for mouse tissues and 2863×2454 pixels for human tissues.

3 Use a manual RGB-based color thresholding algorithm for mouse tissues to exclude pure methyl green nuclei staining from the measurement. For human tissues, use NIS-elements (version 5.10.01, https://www.microscope.healthcare.nikon.com/products/software/nis-elements, RRID:SCR_014329) auto-thresholding algorithm.

4 Export the percentage area of the thresholded signal (object area fraction provided in the program) and normalize to the average value of non-CIAP treated tissues.