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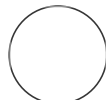
## TruAI Neuromelanin Quantification

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### ABSTRACT

Quantification of area and optical density of intracellular neuromelanin with TruAI.

## Loading training label function in TruAI software

- 1 Open scanned images with Olympus VS200 Desktop (EVIDENT Technology GmbH, ver. 4.1.1 build 29408).
- 2 Under the 'Detect' window, select 'Training Labels'.

## Creating NM foreground training label class

- 3 Create a new training label class by selecting the star icon.
- 4 A new foreground class will appear under level 1.
- 5 Rename as 'NM' referring to neuromelanin.

## Optimize background and NM training label class

- 6 Under the automatically created 'background' level, select the fill icon and outline an area of the section with approximately 50-100 NM granules. The outlined area must be continuous.
- 7 Select the 'NM' class and use the same fill option to outline the shape of each NM granule as closely as possible.
- 8 To ensure maximum accuracy of the neural training, NM granules of all sizes and densities should be drawn.

- 9 Further, all NM granules in each background area should be drawn.
- 10 Save and export this NM training class set and apply it to 5-10 scanned sections.
- 11 Repeat steps 1-5 identically for each image.

## Deep learning training

- 12 In the 'Deep Learning' window, select 'New Training' and pick 'Image Segmentation' option.
- 13 In the 'New Training: Input and Output' pop-up window, load all images used in the 'Optimize background and NM training label class' section.
- 14 Ensure the input channel is RGB.
- 15 Select 'Specific Network (RGB)' under 'Training Configuration'.
- 16 Start training and run until at least 0.85 similarity is reached.

## Applying NM neural network to the scanned sections

- 17 After successful completion of deep learning training, open a scanned brightfield section with Olympus VS200 Desktop (EVIDENT Technology GmbH, ver. 4.1.1 build 29408).
- 18 In the 'Detect' window, select the 'Count and Measure' drop down menu and pick the 'New ROI' option to create ROIs for further anatomical delineation.
- 19 Once all the ROIs have been drawn, select the 'Neural Network Segmentation' option above.
- 20 In the 'Neural Network Segmentation' pop-up window, load the saved NM neural network and adjust the 'Detection threshold' to 0%.
- 21 Proceed by selecting 'Count and Measure on ROI'.

## Thresholding and analysis of intracellular NM granules

- 22 The generated results appear in the 'Count and Measure Results'.
- 23 The corresponding ROI for each NM granule can be found in the 'ROI' column. Other computed parameters relevant to size and intensity of NM are also listed, eg. 'Area  $\mu\text{m}^2$ ', 'Mean (Color Intensity Value)', 'Mean (Saturation)', and 'Mean (Hue)'.
- 24 To identify the intracellular NM population from the total NM granules detected, the modality of the area distribution was used.

It was determined that minimum  $73 \mu\text{m}^2$  was the threshold area for intracellular NM and was used as the cut-off in this analysis.