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TruAI Neuromelanin Quantification

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ABSTRACT

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

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

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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 μm^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.

25 It was determined that minimum 73 μm^2 was the threshold area for intracellular NM and was used as the cut-off in this analysis.