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

Quantification of area and optical density of intracellular neuromelanin with TruAI in H&E stained sections

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ABSTRACT

Quantification of area and optical density of intracellular neuromelanin with TruAI in H&E stained sections

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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 27564).

2 Under the 'Detect' window, select 'Training Labels'.

Creating training label classes

3 Create a new training label class by selecting the star icon. Change the class name and the color by double click on it.

4 A new foreground class will appear under level 1.

5 Rename as 'NM' referring to intracellular neuromelanin.

6 Repeat steps 3-5 for all the label classes: extracellular NM, Non-pigmented cells and background.

7 Once you have added all your classes you have to save the set of training classes to use it in several images. It is very important that the classes you use in different images have exactly the same names and colors and the easy way to do it is by saving the set.

8 Once you have the set you can load it in all your images of interest by clicking the **folder icon** once the image is opened.

Manage sets of training label classes

9 The **hand icon** lets you manage the label sets: here you can export a label class to be able to import it in other computer. This is interesting to export a set of label classes from the workstation to your own computer. As we said before, to have exactly the same label classes is very important to analyze a set of images because, if not, the algorithm will not be correctly processed.

Optimize background and all training label classes

10 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.

11 Select the 'NM' class and use the same fill option to outline the shape of each NM granule as closely as possible.

12 To ensure maximum accuracy of the neural training, NM granules of all sizes and densities should be drawn.

13 Further, all NM granules in each background area should be drawn.

14 Save and export this NM training class set and apply it to 5-10 scanned sections.

15 Repeat steps 11-14 identically for each image and for each training class.

Deep learning training

16 In the ‘Deep Learning’ window, select ‘New Training’ and pick ‘Image Segmentation’ option.

17 In the ‘New Training: Input and Output’ pop-up window, load all images used in the ‘Optimize background and all training label classes’ subsection.

18 Ensure the input channel is RGB.

19 Select ‘Specific Network (RGB)’ under ‘Training Configuration’.

20 Start training and run until at least 0.85 similarity is reached.

Applying neural network to the scanned sections:

21 After successful completion of deep learning training, open a scanned brightfield section with Olympus VS200 Desktop (EVIDENT Technology GmbH, ver. 4.1.1 build 27564).

22 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.

23 Once all the ROIs have been drawn, select the ‘Neural Network Segmentation’ option above.

24 In the ‘Neural Network Segmentation’ pop-up window, load the saved neural network and adjust the ‘Detection threshold’ to 0%. Proceed by selecting ‘Count and Measure on ROI’.

Thresholding and analysis of the results

25 The generated results appear in the ‘Count and Measure Results’.
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, e.g. ‘Area μm^2’, ‘Mean (Color Intensity Value)’, ‘Mean (Saturation)’, and ‘Mean (Hue)’.