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Online Resource 1: Step-by-step ImageJ protocol for quantitative assessment of thalamic microvascular lesion size.

Thalamus ImageJ Step-by-Step Guide

In the freely available software RadiAnt, we identified 3–4 T2-weighted sequence slices that pass through the basal ganglia and thalamus. In the next step, the window settings were adjusted to the values 640/1280, which, based on our own experience, provide the optimal contrast for distinguishing white from grey or black image artifacts. The axial T2 sequence was used because it is the most convenient method for examining the thalamus, as the investigated pathology appears with marked hyperintensity on this sequence. Each suitable image was saved in BMP format without significant loss of quality, with a total of three images per patient. ImageJ software was used for further steps. The images were opened and visualized using the "image sequence" function from the "import" submenu (Figure 1) [1].

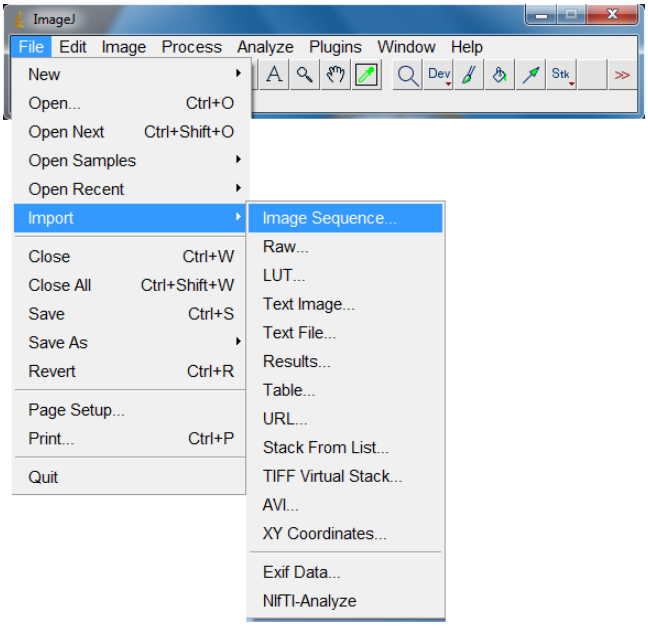

Figure 1. Opening images in ImageJ.

I. Thalamic segmentation

The thalamus was isolated by manual tracing according to the criteria described below.
The anterior boundary, corresponding to the anterior thalamic pole, forms the posterior wall of the interventricular foramen. The medial portion of the thalamus forms the lateral wall of the third ventricle, while cranially it forms part of the floor of the lateral ventricle. Laterally, the thalamus is bordered by the posterior limb of the internal capsule. The posterior end extends to form the pulvinar, which projects beyond the superior colliculus (Figure 2) [2,3].

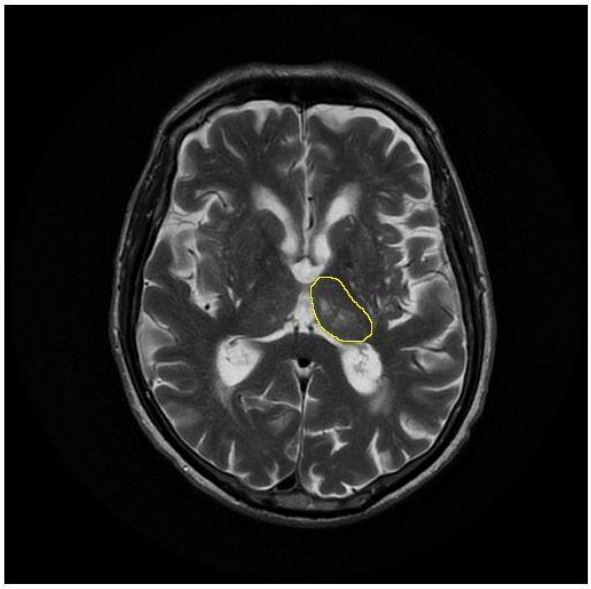

Figure 2. Segmentation of the left thalamus on an MRI image from our case series.

II. Calculation of total thalamic area

The total thalamic area was measured using the "Measure" function in the "Analyse" menu, with the result expressed in pixels. The images were saved in a separate file (Figure 3).

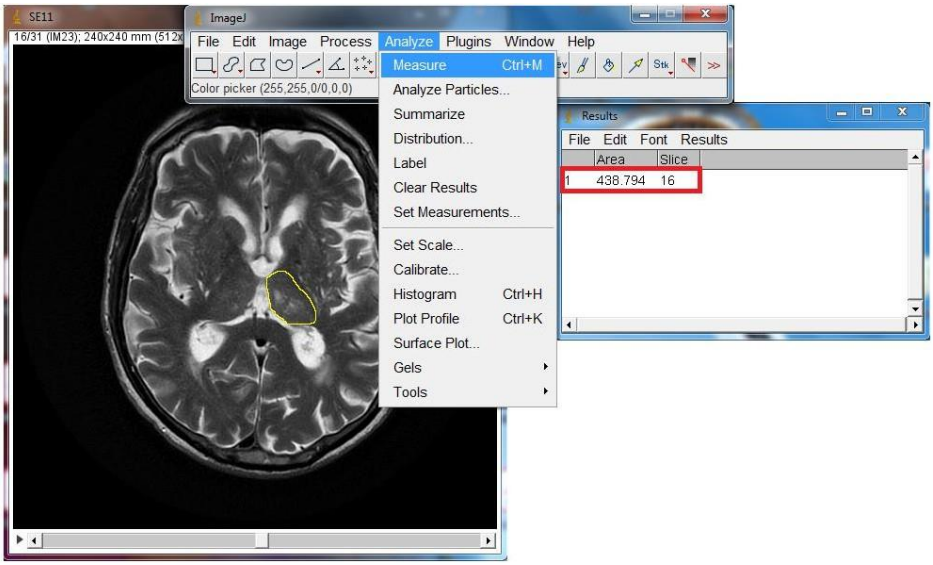

Figure 3. Calculation of total thalamic area using the "measure" function.

III. Calculation of thalamic lesion area

After tracing the thalamic boundaries, the region was opened in a new window using the "duplicate" function. By applying the "clear outside" function, adjacent structures outside the demarcation line were eliminated, so that in the next step, they would not be included in the analysis (Figure 4).

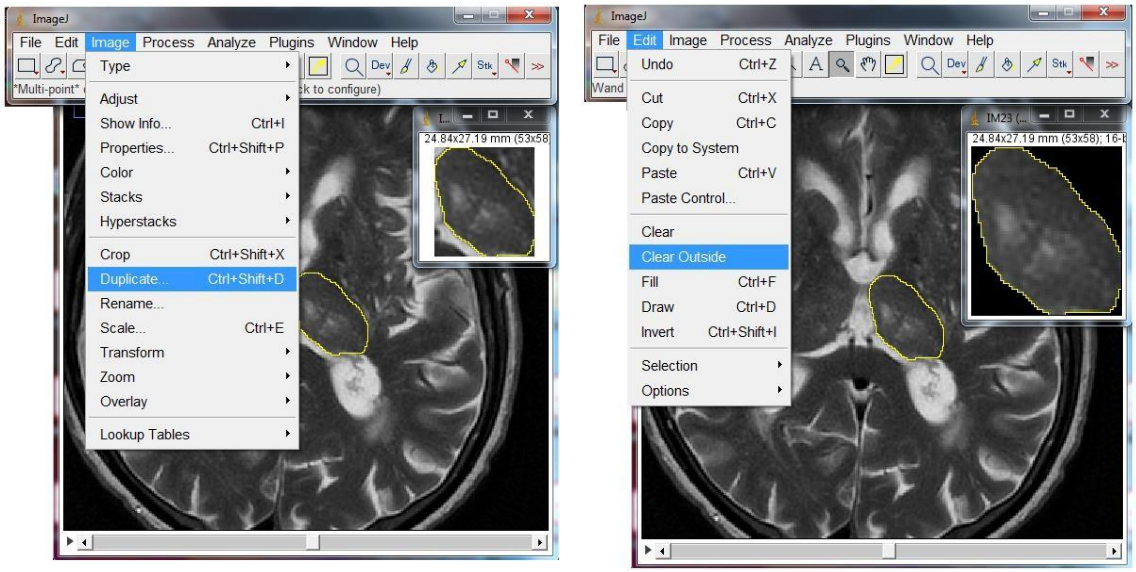

Figure 4. Use of “duplicate” and “clear outside” functions in ImageJ.

After manually adjusting contrast and brightness to highlight lesion areas, we applied the “threshold” function from the “adjust” submenu. In the threshold table, using the arrows (highlighted in the red box in Figure 5), we selected the more hyperintense parts in the T2 sequence, which appeared as formations or small, brighter white territories. After processing, these appeared in red (Figures 5 and 6) [4].

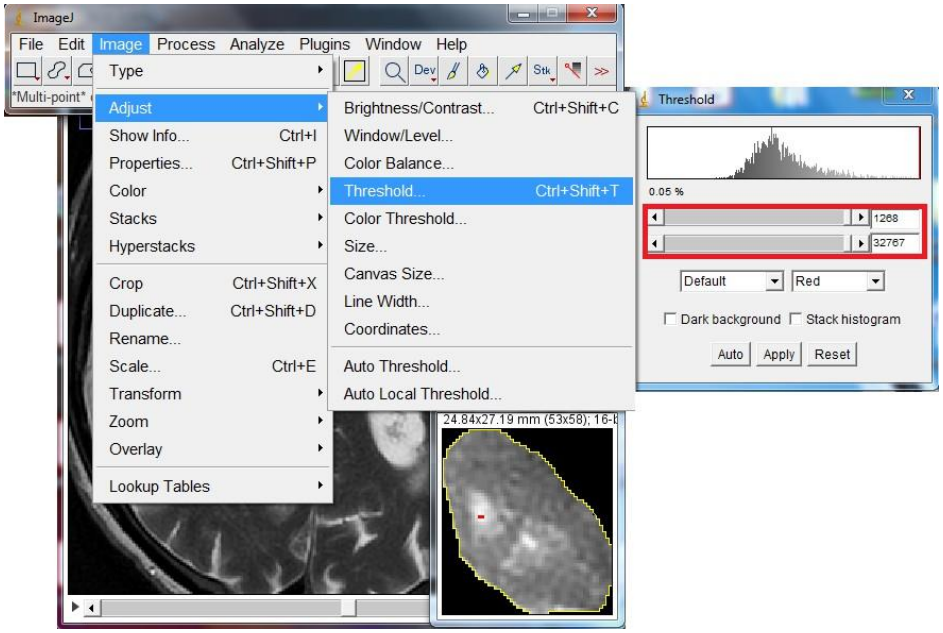

Figure 5. Use of the “threshold” function.

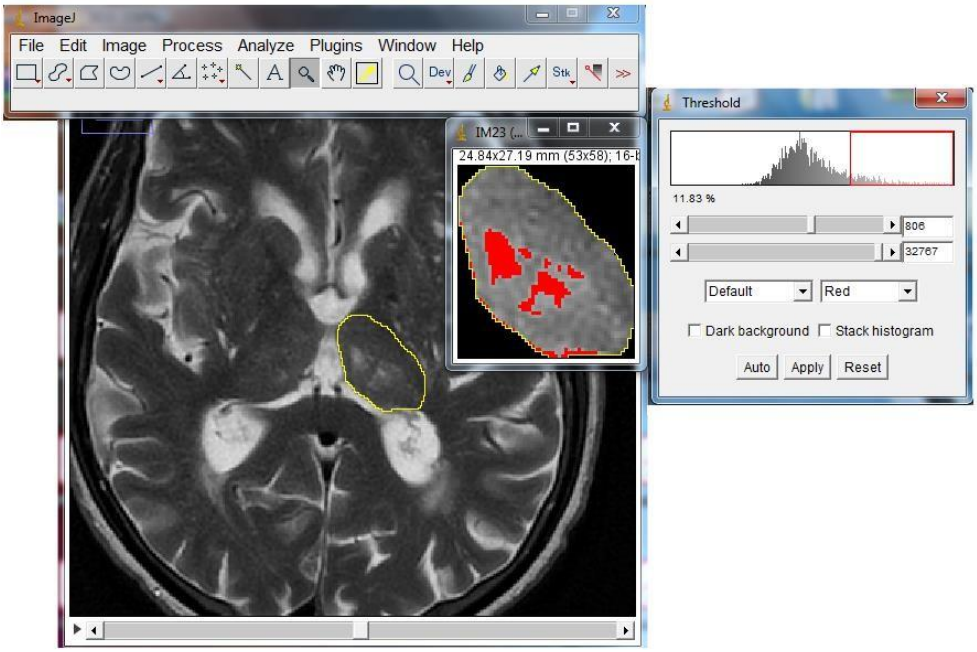

Figure 6. Highlighting hyperintense territories using the arrows in the “threshold” window.

In the figure below, the thalamus can be seen before and after applying the “threshold” function.

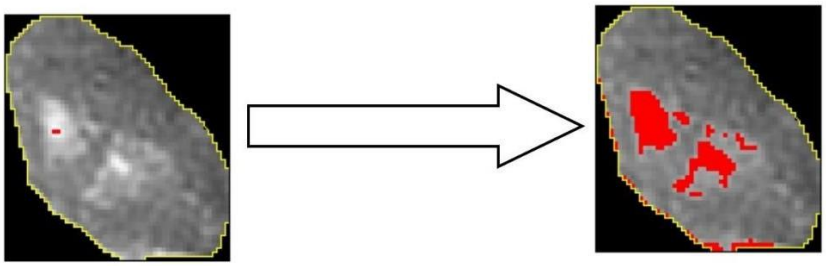

Figure 7. The thalamus before and after applying the threshold function.

For the calculation of the thalamic lesion area, we measured the hyperintense area (also expressed in pixels), now highlighted in red, again using the “measure” function. To limit the calculation only to the modified area, we had previously set the “limit to threshold” option in the “set measurements” box (Figures 8 and 9). These settings and threshold values were kept constant among images from our case series.

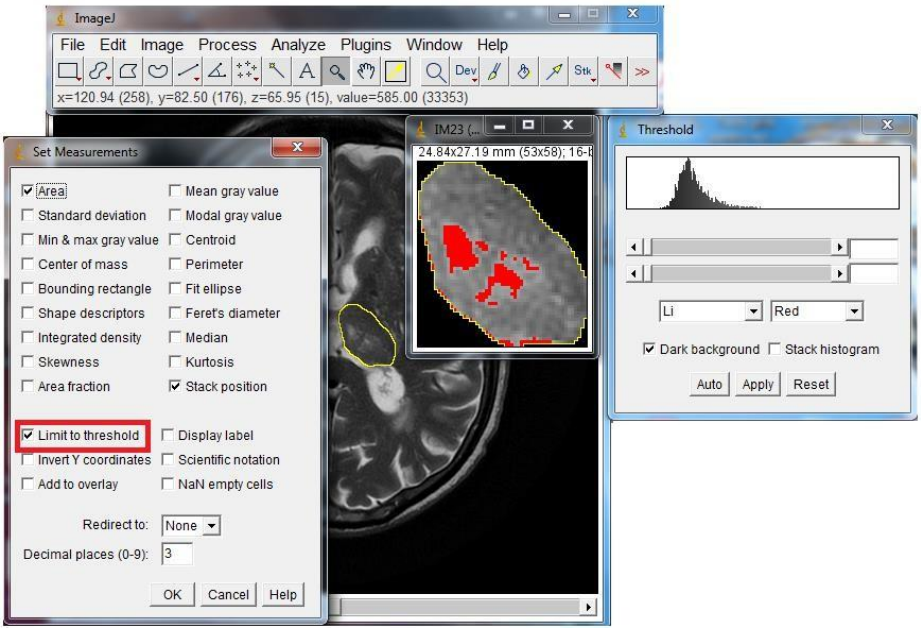

Figure 8. Selecting the "limit to threshold" option to restrict calculation of the thalamic area only to the modified region.

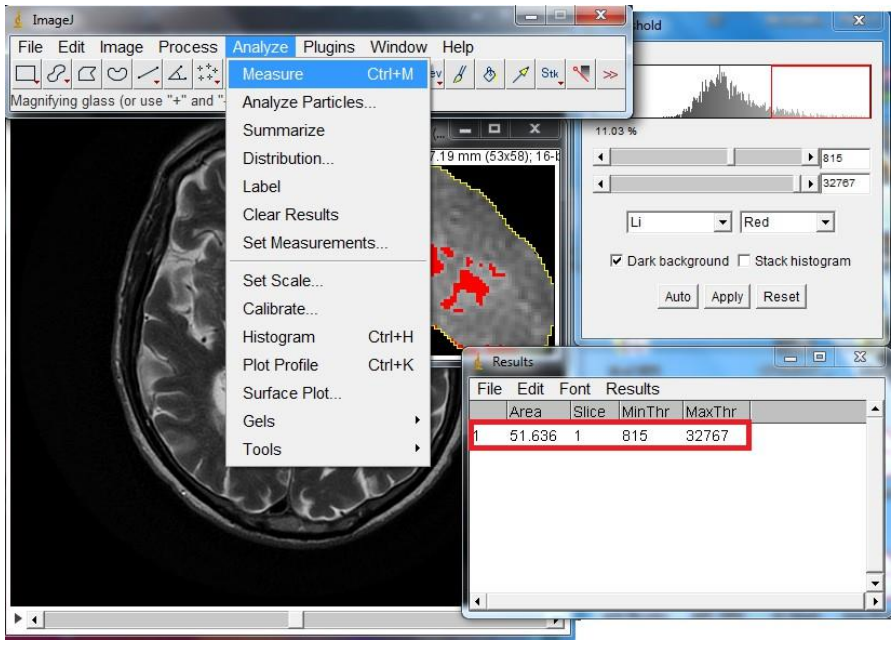

Figure 9. Calculation of thalamic lesion area using the "measure" function.

These procedures were repeated for both sides (left–right) in each patient, followed by calculation of the total thalamic areas and the corresponding hyperintense lesion areas. The final thalamic microvascular lesion size (TML size, %) was calculated as the percentage of total thalamic area occupied by hyperintense lesion area. The data were then saved and processed in a Microsoft Excel file.

References:

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