## Processing algorithm by code inspection at 1.4.3

1. Colour deconvolution to separate H and DAB
2. For H:
   1. Threshold at fixed level (192)
   2. Make binary image
   3. Clean with morphological close and open: remove isolated pixels or holes.
   4. Watershed to split touching bodies.
   5. Find roundish (circularity 0.5-1) blobs within size range 100-600 to mark cell bodies/soma.
   6. Create a mask
3. For DAB:
   1. Threshold at auto level (Huang)
   2. Make binary image
   3. Clean with morphological close
   4. Find particles with size > 200. Should be microglia.
   5. Measure overlap of each process with body
4. Post processing: For each process ROI
   1. Discard process ROIs where process does not overlap any soma (overlap less than <100)
   2. Collate properties (Feret diam, area, overlap with soma area, position)
   3. Run Sholl analysis and collect max branches and mean branches for each process
5. Save results.

An ImageJ macro identifies nuclei (stained with haematoxylin, blue) and microglia (stained with DAB, brown). We keep only those microglia that have an identified nucleus within them (to exclude ‘orphaned’ processes protruding into the slice from other slices either side). We calculate the position, total area, Feret diameter and the maximum and mean number of branches (using Sholl analysis) for each microglia.

We then post-process the position data to find the nearest neighbour distance for each microglia as a way of measuring the evenness of their distribution.

Don’t know if you are using the nearest neighbour matlab calculations

Feret diameter can be defined if you want.