**S2: IMARIS Algorithms for segmentation (Cortex, striatum, thalamus):**

*Imaris algorithms for surface rendering of microglia (surface 1), NeuN labeled neurons (surface 2) and surfaces of vgat-positive and vglut-positive neurons (surface 3) in the cortex, striatum and thalamus (fig. 2 and fig. 3). Thresholds, “quality” to classify seed points and “number of voxels” to classify the surfaces were adjusted manually for each dataset. The values (in brackets) are approximate values taken from one example, and can slightly vary between the samples.*

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| --- | --- | --- |
| Surface 1 (microglia) | Surface 2 (NeuN) | Surface 3 (vgat, vglut) |
| Enable Region Of Interest = false  Enable Region Growing = false  Enable Tracking = false  [Source Channel]  Source Channel Index = 3  Enable Smooth = true  Surface Grain Size = 0.900 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 8.00 um  [Threshold]  Enable Automatic Threshold = false  Manual Threshold Value =( 48.1402)  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = (199.588)  Active Threshold B = false  [Classify Surfaces]  "Number of Voxels Img=1" above (500) | Enable Region Growing = true  Enable Tracking = false  [Source Channel]  Source Channel Index = 2  Enable Smooth = true  Surface Grain Size = 1.00 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 10.0 um  [Threshold] absolute Intensity  Enable Automatic Threshold = false  Manual Threshold Value = (28.5981)  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = (195.213)  Active Threshold B = false  Region Growing Estimated Diameter = 5.00 um  [Classify Seed Points]  "Quality" above (31.7)  [Classify Surfaces]  "Number of Voxels Img=1" above (650) | Enable Region Growing = true  Enable Tracking = false  [Source Channel]  Source Channel Index = 4  Enable Smooth = true  Surface Grain Size = 0.900 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 10.0 um  [Threshold]  Enable Automatic Threshold = false  Manual Threshold Value = (21.8303)  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = (228.49)  Active Threshold B = false  Region Growing Estimated Diameter = 5.00 um  [Classify Seed Points]  "Quality" above (13.9)  [Classify Surfaces]  "Number of Voxels Img=1" above (560) |