Processing Steps for Supplementary Data 3.

Supplementary Text (Suppl. Data 4) for
"Automated Tracing of Filaments in 3D Electron Tomography Reconstructions using *Sculptor* and *Situs*" by
Mirabela Rusu, Zbigniew Starosolski, Manuel Wahle, Alexander Rigort, and Willy Wriggers

We list the processing steps for the validation sub-volume (Suppl. Data 3) used the main text. The mentioned software tools correspond to *Sculptor* version 2.1 and *Situs* version 2.7 at `http://sculptor.biomachina.org` and `http://situs.biomachina.org`.

1. *Sculptor*: scale by -1;
2. *Sculptor*: threshold densities to [-2, 2];
3. *Sculptor*: 'normalize' (scale and shift) densities to [0, 1];
4. *Sculptor*: apply Gaussian with sigma-1D = 1 voxel (19.12 Å), kernel cutoff 3 sigma-1D;
5. *Sculptor*: apply local normalization with Gaussian sigma-1D = 10 voxels, kernel cutoff 2 sigma-1D;
6. *Sculptor*: 'normalize' (scale and shift) densities to [0, 1];
7. *Situs* - *voledit*: polygon cropping with 6 vertices (1 200; 200 200; 200 1; 126 18; 34 74; 1 104);
8. *Sculptor*: apply multi-point floodfill for 20 restarts, threshold value 0.475, Gaussian sigma-1D = 2 voxels, kernel cutoff 3 sigma-1D;
9. *Sculptor* - *Lua*: mask raw map at threshold 0.01 with the outcome of multi-point floodfill;
10. *Situs* - *voldiff*: substract densities under mask.