Supplementary Information for:

Dense neuronal reconstruction through X-ray holographic nano-tomography

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Supplementary Data

Supplementary Data Tables

Sample	Voxel Size (nm)	Scan Edge Length (μm)	Scan Edge Length, Ext FOV (μm)	Sample Size (μm)	Measured Resolution (nm)	Measured Resolution ÷ Scan Voxel Size	Num. subvolumes for resolution measurement
mouse cortex	30	61	96	300 x 200 x 1000	87 [-4, +5]	2.9 [-0.1, +0.2]	125
mouse cortex	40	82	129	300 x 200 x 1000	131 [-5, +6]	3.3 [-0.1, +0.2]	125
mouse cortex	40	82	129	300 x 200 x 1000	134 [-3, +5]	3.3 [-0.1, +0.1]	125
Drosophila VNC	50	102	161	200 x 200 x 600	139 [-5, +5]	2.8 [-0.1, +0.1]	89
Drosophila brain	50	102	161	300 x 200 x 700	174 [-11, +10]	3.5 [-0.2, +0.2]	125
Drosophila leg	60	122	192	200 x 200 x 600	186 [-2, 14]	3.1 [-0.0, 0.2]	110
Drosophila leg	75	154	241	400 x 600 x 800	198 [-8, +10]	2.6 [-0.1, +0.1]	11
Drosophila leg	80	164	257	200 x 200 x 600	197 [-7, 17]	2.5 [-0.1, 0.2]	116
Drosophila leg	100	205	322	200 x 200 x 600	215 [-7, 20]	2.1 [-0.1, 0.2]	4
Drosophila brain	100	205	322	300 x 200 x 700	222 [-9, +10]	2.2 [-0.1, +0.1]	4
Drosophila brain	120	246	386	300 x 200 x 700	183 [-8, +16]	1.5 [-0.1, +0.1]	8

Supplementary Data Table 1: List of XNH scans included in resolution quantification (Figs. 1h, Extended Data Fig. 1b). "Ext FOV" refers to extended field of view reconstructions (see Methods). Resolutions are reported as median [interquartile range] of sampled subvolumes.

Scan number	Contents	Voxel Size (nm)
1	Overview of VNC T1s	160
2	VNC, left T1	50
3	Overview centered on VNC left T1 nerve and top of coxa	160
4	left T1 nerve and top of coxa	75
5	Middle of coxa	75
6	Bottom of coxa and whole trochanter	75
7	Bottom of femur	75
8	Lower-middle of femur	75
9	Middle of femur	75
10	Upper-middle of femur	75
11	Femur-tibia joint	75
12	Upper 2/3rds of tibia	100

Supplementary Data Table 2: List of XNH scans included in the *Drosophila* leg dataset (Fig. 3). Ten high-resolution scans (50-75 nm voxels) comprise the main dataset, while two overview scans (160 nm voxels) were used to enable accurate targeting of higher-resolution scans and to give extended anatomical context.

	Receptor type	Receptor location	Cluster name	# receptors in cluster	Comparison with previous literature	Nerve of entry into VNC
Surface structures	Hair plates	Thorax-coxa joint	CoHP3	3	Consistent with [1]	Dorsal
			CoHP4	4	Consistent with [1]	Accessory
			CoHP8	8	Consistent with [2] but 2 more than in [1]	Ventral
		Coxa-trochanter joint	TrHP1	1	Consistent with [2] but not reported in [1]	Main
			TrHP5	5	Consistent with [1]	Main
			TrHP6	6	Consistent with [1]	Main
			TrHP7	7	Consistent with [1]	Main
		Femur-tibia joint	TiHP3	3	2 more than reported in [2]	Main
	Campaniform sensilla	Trochanter	TrCS3	3	Consistent with [1]	Main
			TrCS5	5	Consistent with [1]	Main
			TrCS8	8	Consistent with [1]	Main
		Femur	FeCS1	1	Consistent with [1]	Main
			FeCS11	11	Consistent with [1]	Main
		Tibia	TiCSd2	2	Consistent with [1]	Main
			TiCSv1	1	Consistent with [1]	Main
			TiCSv2	2	Consistent with [1]	Main
	Bristles	Coxa	N/A†	13	Qualitatively similar to previous reports. Total numbers could not be found in previous reports.	Unresolved
		Trochanter	N/A†	10		Main
		Femur	N/A†	113		Main
		Tibia	N/A†	97*		Main
Internal structures	Chordotonal scolopidia	Femur	FeCO	76 (152 neurons‡)	More than range of 100-138 neurons reported in [3]	Main
	Stretch receptor neurons	Coxa	N/A†	1	Not labeled in [4]	Ventral
		Femur	N/A†	1	Consistent with [4]	Main
	Strand receptors	Coxa	N/A†	1	Only reported in orthopteran insects [5]	Accessory

Supplementary Data Table 3: Sensory receptors identified in an XNH dataset of a *Drosophila* front leg (Figs. 3, Extended Data Fig. 3). Gray cells contain information that modifies or extends reports from previous literature. For nerves of entry, "Main" refers to the prothoracic leg nerve; "Dorsal" refers to the dorsal prothoracic nerve; "Ventral" refers to the ventral prothoracic nerve; "Accessory" refers to the prothoracic accessory nerve[6]. *Excludes bristles on the bottom half of the tibia, which was not imaged. †Bristles, stretch receptors, and strand receptors are not organized into clusters. Reported numbers are total counts per leg segment. ‡We find that each scolopidium of the femoral chordotonal organ has the dendrites of two sensory neurons associated with it.

Descriptions of Supplementary Videos

Supplementary Video 1: An XNH scan of an adult *Drosophila* brain at a voxel size of 120 nm and a measured resolution of 183 nm. At this resolution, many individual neurons can be tracked as they travel between different brain regions. The field of view encompasses the entire central brain and part of both optic lobes, allowing brain-wide projections to be mapped.

Supplementary Video 2: An XNH scan of mouse somatosensory cortex (layer 5) at a voxel size of 30 nm and a measured resolution of 87 nm. At this resolution, all myelinated axons and most unmyelinated axons and dendrites are resolved. Many subcellular features, most notably mitochondria and endoplasmic reticulum, are resolved.

Supplementary Video 3: An XNH scan of the adult *Drosophila* VNC, encompassing the T1 neuromere that controls movements of a front leg. This single scan at 50 nm voxels captures the majority of the T1 neuromere with sufficient resolution (139 nm) to reconstruct single neurons and identify different types of sensory and motor neurons (see Fig. 4). Body wall muscles surrounding the neuromere, as well as the motor neurons innervating those muscles, are also visible.

Supplementary Video 4: An XNH scan of part of the adult *Drosophila* leg at a voxel size of 75 nm and a measured resolution of 198 nm. The inset shows a zoom-in on the leg nerve, which contains motor neurons (large diameter axons) traveling out to muscles, as well as sensory neurons (variable diameter depending on type, see Fig. S3) bringing mechanosensory information to the central nervous system. The structural arrangement of muscles, fats, and tendons, and joints are visible (see Fig. 3).

Supplementary Video 5: Automated segmentation of a portion of the adult *Drosophila* VNC encompassing the T1 neuropil (same data as Supplementary Video 3 and Fig. 4). Each neuron is rendered in a color corresponding to neuron types determined based on 3D morphology (as in Fig. 4c). Blue: motor neurons; Orange: campaniform sensillum neurons; Purple: hair plate neurons; Red: chordotonal organ neurons; Green: bristle neurons.

Supplementary Video 6: An XNH scan at 105 nm voxel size of an adult *Drosophila* brain prepared without any heavy metal staining. Phase-contrast imaging enables this type of soft tissue to be imaged with reasonable contrast. As with stained samples (Videos 1-4), many individual neurons can be reconstructed traveling within and between brain regions.

Supplementary References

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