Cell Centered Database
University of California, San Diego
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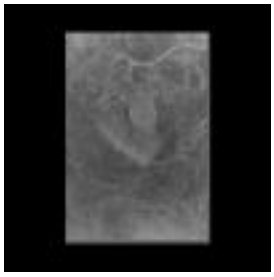
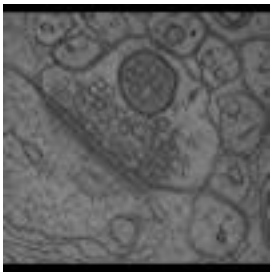
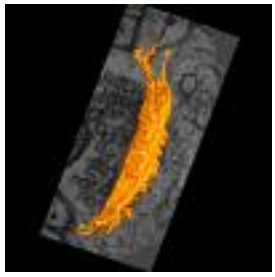
Microscopy Product #:3684 HPFcere2

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=3684

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1243
PROJECT_NAME	High Pressure Freezing and Freeze Substitution
PROJECT_DESCRIPTION	This project is designed to achieve ultimate ultrastructure of animal tissues.
LEADER	Mark Ellisman, Gina Sosinsky, Ying Jones
FUNDING_AGENCY	NIH
PROJECT_START_DATE	2004-01-01 00:00:00.0
PROJECT_END_DATE	
COLLABORATORS	
PUBLICATION1	Sosinsky GE, Crum J, Jones YZ, Lanman J, Smarr B, Terada M, Martone ME, Deerinck TJ, Johnson JE, Ellisman MH. The combination of chemical fixation procedures with high pressure freezing and freeze substitution preserves highly labile tissue ultrastructure for electron tomography applications. J Struct Biol. 2007 Sep 14; PMID: 17962040
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	To achieve better ultrastructure of brain and nerve tissue.
TITLE	HPF/FS on fixed rat brain and nerve tissues of rat
EXPERIMENTER	Ying Jones
EXPERIMENT_NAME	
EXPERIMENT_DATE	2006-08-17 00:00:00.0

Subject Information -	
GROUP_BY	Type of fixation
SUBJECT_NAME	CAF-HPF
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	rat
SPECIES	rat sprague dawley
STRAIN	sprague dawley
AGE	21 days
AGECLASS	young adult
ANIMAL_NAME	
LITTER_ID	
SEX	male
VENDOR	
WEIGHT	grams

Tissue -	
ANATOMIC_LOCATION	cerebellum
MICROTOME	Ultramicrotome
ORIENTATION	sagittal
THICKNESS	.5 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	Chemical Fixation/High Pressure Freezing

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	3684
IMAGE_BASENAME	HPFcere2
CREATE_DATE	
INSTRUMENT	JEOL 4000#1
MICROSCOPE_TYPE	IVEM
PLANE_COUNT	
PRODUCT_TYPE	DOUBLE TILT
PURL	
SESSION_NAME	
TELESCIENCE_SRB	
X_RESOLUTION	nm/pixels
Y_RESOLUTION	nm/pixels
XSIZE	2242
YSIZE	3340

Protocol:

1. Fixation: A 21 day old rat was perfused with a solution of 2% of paraformaldehyde/2.5% of glutaraldehyde in 0.15 M cacodylate buffer according to the protocol described in (Giepmans, 2005 see original publication for details). Brain was taken out and post-fixed in same fixative for 2 hrs at 4¿C. For brain tissue, 100mm thick sections were kept in the fixative solution until HPF.

2. High Pressure Freezing: Brain sections were cut with 1.8 mm tissue puncher. This step ensures that the proper size of brain tissue will fit into the shallow side of a 100 mm-deep well in the type A HPF brass planchette. For peripheral nerve tissue, the nerves were carefully trimmed to a proper length in order to fit into the type A freezing hats. Trimmed brain or nerve tissue was loaded into the planchettes and the well was filled with 1-hexadecene. The planchette was then covered with the flat side of brass type B planchette, quickly loaded into a freezing holder and frozen with the Bal-Tec HPM 010.

Freeze-substitution procedure: After freezing, the planchette sandwiches were separated under liquid nitrogen and the specimen/type A hats were placed into cryo-vials and stored under liquid nitrogen or subsequently placed into the freeze substitution device. The first substitution media was a solution of filled with freshly made 0.1% tannic acid (EM grade, from Polysciences Inc., Warrington, Pennsylvania) in acetone (EM grade from Fullam Inc., Latham, New York). After 24 hours, samples were then washed three times in cold acetone over a 2 hour period. The solution was changed to 2% osmium tetroxide in acetone for 48 hrs. The temperature was slowly raised to 20 deg C. The specimens were rinsed three times at room temperature for 10 minutes in acetone. Tissues were removed from the planchettes after the last wash step. The total time for this procedure is 113 hrs.

Infiltration and embedding: Infiltration was conducted over 3 days followed by embedding in Durcupan ACM resin (Electron Microscopy Science Inc., Hatfield, PA). Samples were infiltrated in 30% Durcupan in acetone for 4 hours and 50% Durcupan overnight. The next day, the specimens were placed into 70% Durcupan for 4 hours, 90% over 2 hours and were placed in 100% Durcupan for overnight incubation. After two incubations in fresh 100% Durcupan, the sample was then polymerized at 60 deg C for 2 days.

Specimen Preparation Information:

Specimen Description -	
ANATOMICAL_DETAIL	6165
ATLAS_COORD	, ,
ORGAN	brain
REGION	cerebellum
STRUCTURE	synapse
SYSTEM	central nervous

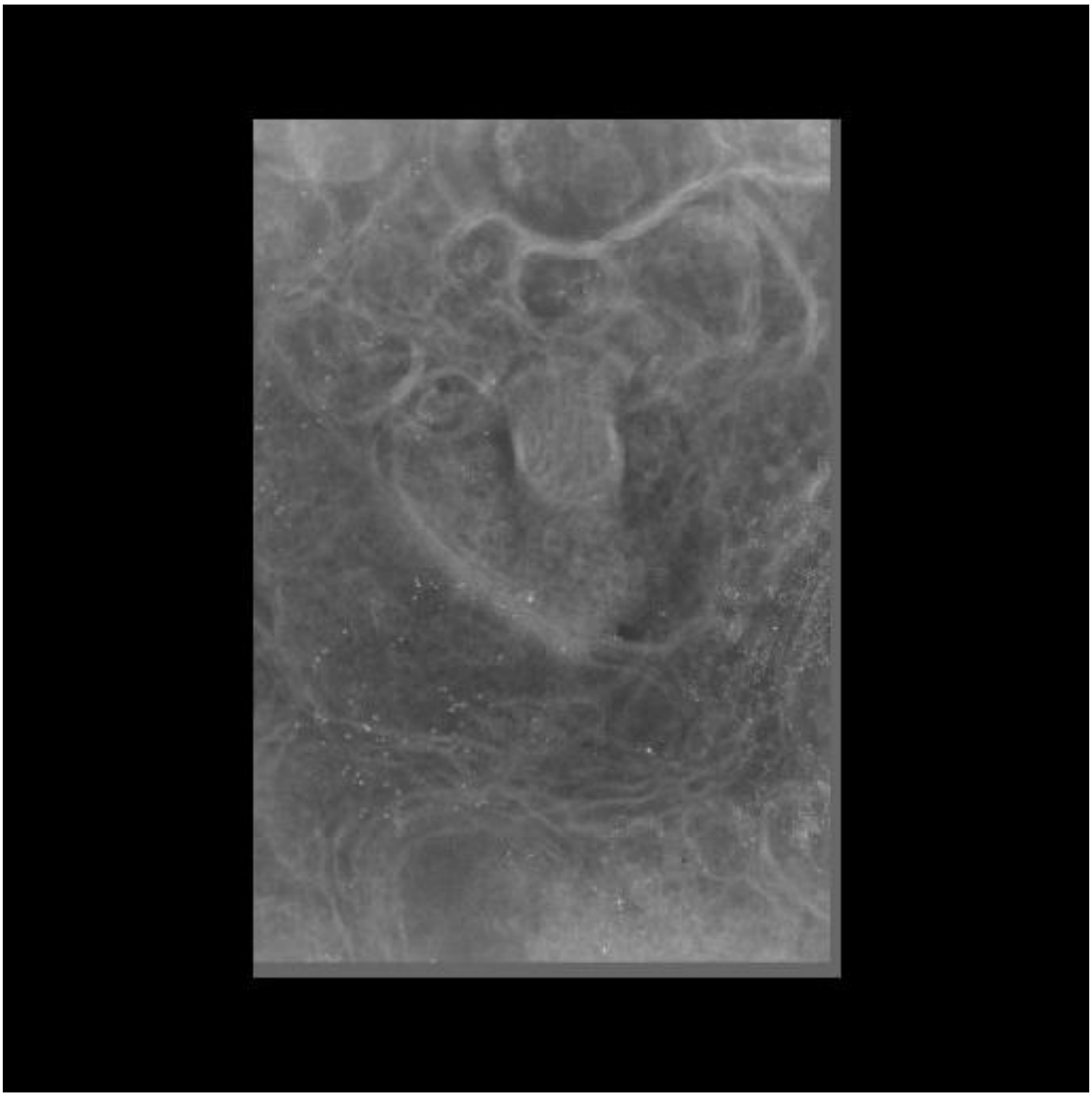
Imaging Parameters:

Image Type -	
DOUBLETILTIMAGESEQ_ID	6072
DOUBLET_DESC	double-tilt tomographic dataset of high pressure frozen cerebellum tissue
RANGE_MAX_X	60 degrees
RANGE_MAX_Y	60 degrees
RANGE_MIN_X	-60 degrees
RANGE_MIN_Y	-60 degrees
DOUBLET_DESC	double-tilt tomographic dataset of high pressure frozen cerebellum tissue
TILT_INCREMENTX	2degrees
TILT_INCREMENTY	2 degrees

Electron Microscopy Product -	
EM_PRODUCT_ID	6165
ACCELERATING_VOLTAGE	400 kV
EMBEDDING_MEDIUM	resin
MAGNIFICATION	50000
RECORDING_MEDIUM	film

Raw 2D Image

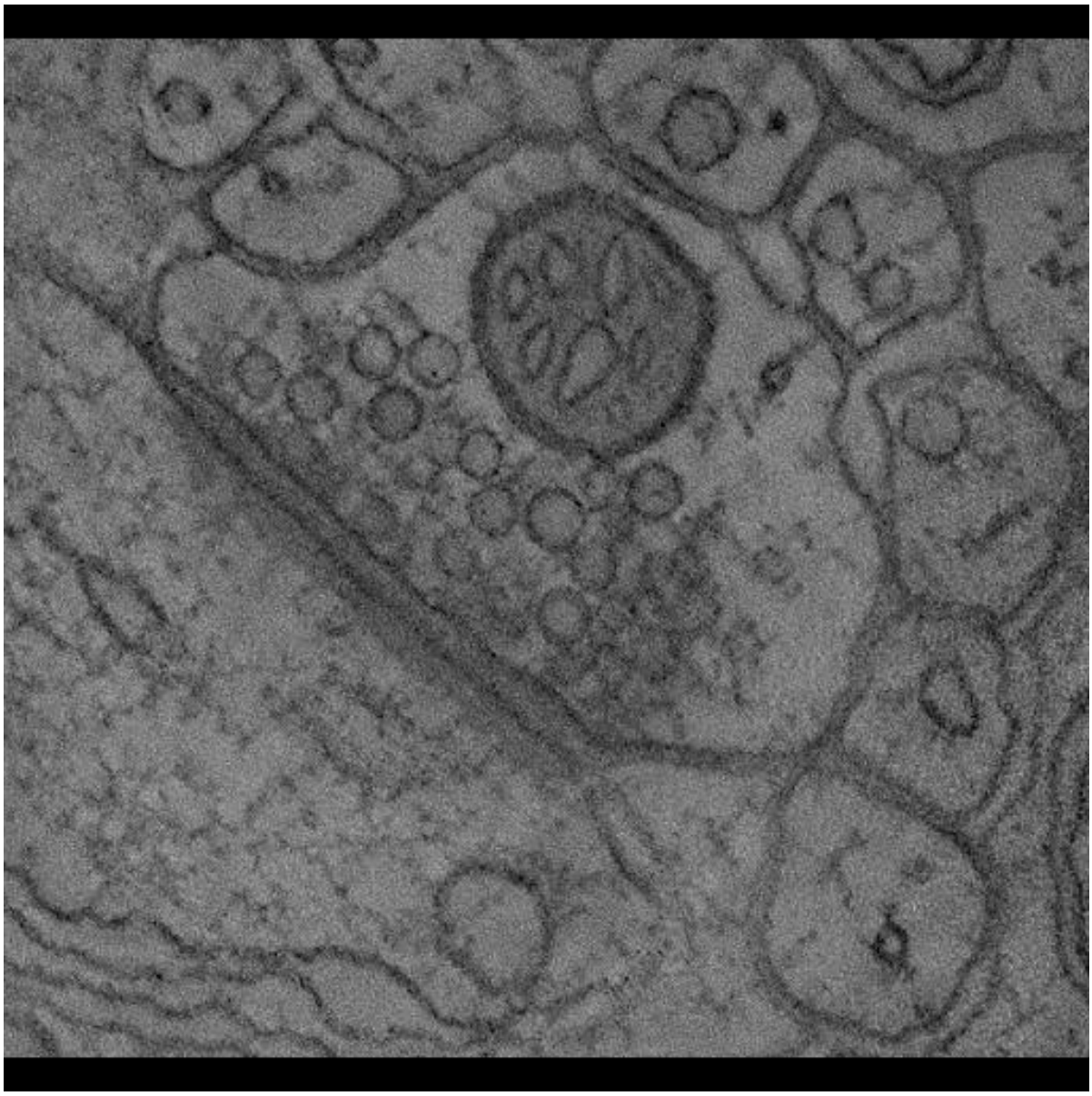
Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	6148
BIT_DEPTH	16 bit
DIGITIZED_BY	Masako Terada
DIGITIZING_PLATFORM	Nikon SuperCool Scan 9000ED
IMAGE_DESC	Tar file containing original digitized TIFF images (in folder marked A and B), IMOD files (*com, *log, st, preali, fid, rawtlt,...), and TxBR files (*mat, *txt, preali, rawtlt, fid, ...) of high pressure frozen Cerebellar tissue, showing post synaptic density. Note total file size is ~ 11 Gb, so this will take a long time to download.
IMAGE_FILE_FORMAT	imod
IMAGE_FILE_NAME	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/HPFcere2a_img.jpg
MAGNIFICATION	50000 X
RAW_ANIMATION_DESC	Aligned tilt series (one of two orthogonal tilt series from a double tilt tomogram) of a spine synapse from the molecular layer of cerebellar cortex tissue prepared using a combination of conventional chemical fixation followed by high pressure freezing, imaged using intermediate voltage electron microscopy.
RAW_ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1243/Experiment_3396/Subject_109/Tissue_161/Microscopy_3684/HPFcere2a_img.mpg
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1243/Experiment_3396/Subject_109/Tissue_161/Microscopy_3684/HPFcere2_img.tar
THUMBNAIL_DESC	Zero degree tilt electron micrograph from a double tilt series of a spine synapse from the molecular layer of cerebellar cortex tissue prepared using a combination of conventional chemical fixation followed by high pressure freezing, imaged using intermediate voltage electron microscopy
THUMBNAIL_FILE	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/HPFcere2a_img_thmb.jpg
X_RESOLUTION	.0005 um/pixel
Y_RESOLUTION	.0005 um/pixel
X_SIZE	2242 pixels
Y_SIZE	3340 pixels

Reconstruction

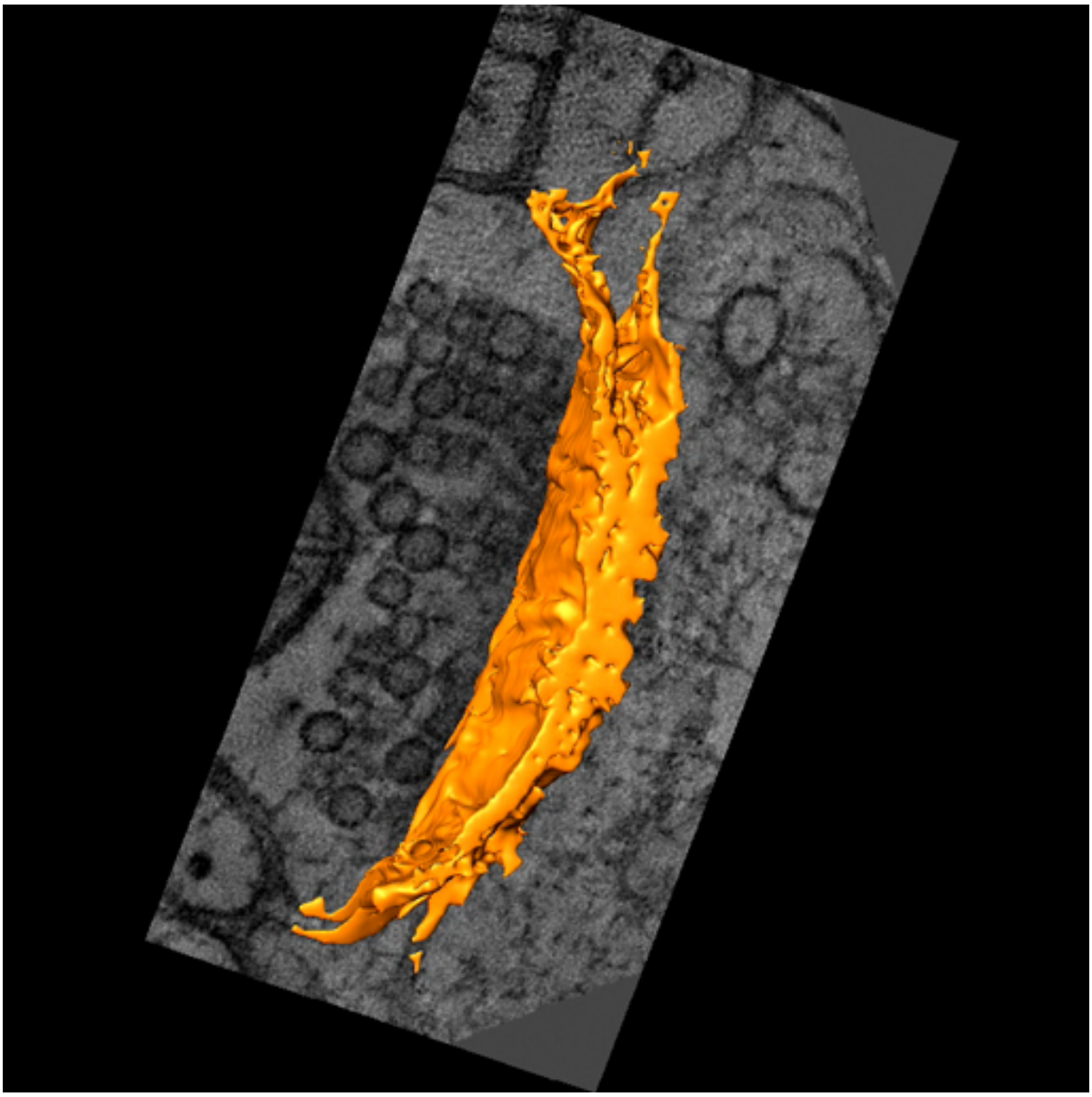
Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	15298
ALIGNMENT_METHOD	Imod
ALIGNMENT_PROGRAM	IMOD
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
RECON_ALGORITHM	R-weighted back projection
RECON_DESC	Tar archive file containing TxBR combined (double tilt) tomographic volume of high pressure frozen cerebellar tissue in IMOD format. Two versions of the volume are included: the full resolution version HPFcere2_full.rec; a trimmed version HPFcere2_proj_trim.rec and a trimmed downsampled version HPFcere2_proj_trim_rot_trim_bin2.rec. Volume dimensions and scales given in CCDB are for the full resolution version. Also included are various animation files that show different types of projections through the volume. Total size of sitx file ~6 Gb.
RECON_PROGRAM	IMOD
RECON_TYPE	double tilt electron tomography
VOLUME_DIMENSION	1839, 1740, 591
VOLUME_NAME	/telescience/home/CCDB_DATA_USER.portal/P1243/Experiment_3396/Subject_109/Tissue_161/Microscopy_3684/HPFcere2_vol.tar
VOXEL_SCALE	.0005, .0005, .0005
RECONSTRUCTION_IMAGES_ID	15299
RECON_IMAGE_DESC	Single computed slice through a tomographic reconstruction of a spine synapse from the molecular layer of the cerebellar cortex from tissue that was prepared from a combination of chemical fixation and high pressure freezing. The contrast was adjusted and the image downsampled to 8 bits from the submitted data for display purposes.
RECON_FILE_NAME	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/HPFcere2_vol.jpg
VOLUME_THUMBNAIL	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/HPFcere2_vol_thmb.jpg
ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1243/Experiment_3396/Subject_109/Tissue_161/Microscopy_3684/HPFcere2_vol.mpg
ANIMATION_FILE_FORMAT	mpg
ANIMATION_DESC	Animation of a tomographic reconstruction of a spine synapse from the molecular layer of the cerebellar cortex from tissue that was prepared from a combination of chemical fixation and high pressure freezing.

Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	18002
ANALYZE_DESC	Isosurfacing of PSD and visualization were performed using Amira v4.1. The PSD was extracted using thresholding.
ANALYZE_DESC	Isosurfacing of PSD and visualization were performed using Amira v4.1. The PSD was extracted using thresholding.
DISPLAY_IMAGE_DESC	Surface rendering of the post synaptic density (orange), viewed within a computed tomographic slice through a synaptic contact in the molecular layer of the cerebellar cortex from tissue that was prepared by a combination of chemical fixation and high pressure freezing.
DOWNLOADABLE_FILE_DESC	Sitx (zipped) archive of segmentation files generated by Amira (.hx, .am, surf) of the post synaptic density. Also included are animation files showing the surfaced PSD (PSDamira_surface_rot_noimage2.mpg) and a the surfaced PSD visualized along with computed slice through the tomographic volume (PSDamira_surface_rot.mpg).
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	1
OBJECT_DESC	post synaptic density
OBJECT_NAME	HPFcere2_psd.hx
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/HPFcere2_seg.jpg
SEGMENT_PERSON_NAME	Masako Terada
SEG_ALGORITHM	simple threshold
SEG_DESC	Segmentation of post synaptic density (PSD) using automatic segmentation
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1243/Experiment_3396/Subject_109/Tissue_161/Microscopy_3684/HPFcere2_seg.sitx
THUMBNAIL	/usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/HPFcere2_seg_thmb.jpg

USER AGREEMENT

Data Sharing and Citation Policy: The mission of the CCDB is to promote data sharing among scientists interested in cellular and subcellular anatomy and in developing computer algorithms for 3D reconstruction and modeling of such data. Data sets may be viewed or shared at the discretion of the author of the data. In some cases, the data may be freely viewed and downloaded without contacting the original author while in other cases, permission of the author may have to be obtained prior to downloading the data. In either case, failure to cite or give proper credit to the original authors who collected these data in subsequent published articles or presentations is a material breach of this User Agreement. CCDB requires all researchers re-analyzing these published data via the CCDB access to reference the original published article and the CCDB. An example of an appropriate acknowledgement is provided on the CCDB web site. CCDB is not in a position to police every intended use of these data. The scientific community will self-police the compliance of this contractual obligation.

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USER NOTIFICATION

For large size image data, it will take several minutes to download, please be patient. Thanks!

ACKNOWLEDGEMENT

Data used from the CCDB should be appropriately referenced, including both the author of the data and the CCDB. If the data were from a published study, the reference is included in the database record. The following reference should be cited for the CCDB:

Martone, M. E., Gupta, A., Wong, M., Qian, X., Sosinsky, G., Ludaescher, B., and Ellisman, M. H. A cell centered database for electron tomographic data. J. Struct. Biology 138: 145-155, 2002.

In addition, the support for the Cell Centered Database should be included in the acknolwedgement section of any publication:

The Cell Centered Database is supported by NIH grants from NCRR RR04050, RR RR08605 and the Human Brain Project DA016602 from the National Institute on Drug Abuse, the National Institute of Biomedical Imaging and Bioengineering and the National Institute of Mental Health, and NSF grants supporting the National Partnership for Advanced Computational Infrastructure NSF-ASC 97-5249 and MCB-9728338.

Maryann Martone