Cell Centered Database

University of California, San Diego

Maryann Martone

Microscopy Product #:22 cere14

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=22

Image2D	Reconstruction	Segmentation

Project Information:

PROJECT_ID	P1134
PROJECT_NAME	Actin in dendritic spines
PROJECT_DESCRIPTION	Actin localization in spines of CNS using eosin phalloidin photooxidation
LEADER	Francisco Capani
FUNDING_AGENCY	NIH
PROJECT_START_DATE	1998-01-01 00:00:00.0
PROJECT_END_DATE	2001-07-31 00:00:00.0
COLLABORATORS	M Martone; M Ellisman
PUBLICATION1	Capani F; Martone ME; Deerinck TJ; Ellisman MH.Selective localization of high concentrations of F-actin in subpopulations of dendritic spines in rat central nervous system:a three-dimensional electron microscopic study.J Comp Neurol.2001 435(2):156-70.
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	The subcellular distribution of F-actin in cerebellar Purkinje cell spines
TITLE	Tomographic reconstruction of phalloidin labeling in cerebellar cortex
EXPERIMENTER	Francisco Capani
EXPERIMENT_NAME	
EXPERIMENT_DATE	1999-10-01 00:00:00.0

Subject Information -	
GROUP_BY	
SUBJECT_NAME	
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	rattus norvegicus
SPECIES	rat
STRAIN	Sprague Dawley
AGE	
AGECLASS	adult
ANIMAL_NAME	
LITTER_ID	
SEX	male
VENDOR	
WEIGHT	

Tissue -	
ANATOMIC_LOCATION	cerebellum
MICROTOME	ultramicrotome
ORIENTATION	sagittal
THICKNESS	1 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	22
IMAGE_BASENAME	cere14
CREATE_DATE	2000-02-01 00:00:00.0
INSTRUMENT	JEOL4000 IVEM
MICROSCOPE_TYPE	IVEM
PLANE_COUNT	
PRODUCT_TYPE	single tilt
PURL	11391638
SESSION_NAME	cere14/cere14_seg.jpg
TELESCIENCE_SRB	P1134/Experiment_14/Subject_14/Tissue_17/Microscopy_22
X_RESOLUTION	.0012 um
Y_RESOLUTION	.0012 um
XSIZE	1024
YSIZE	1024

Protocol:

1) Tissue:

Five male Sprague Dawley adult rats were used in this study. The committee on animal studies of UCSD following the NIH guidelines approved all of experimental

procedures. Briefly, an intracardiac perfusion was performed under deep anesthesia (containing 50 mg/kg ketamine, 1mg/kg

rhompun and 5 mg/kg acetopromazine in
sterile saline) with normal rat Ringer's at 35C followed by fixative. For light microscopic analyses, rats were perfused with 4% formaldehyde (made fresh from
paraformaldehyde) in cacodylate buffer, pH 7.2. The brain was removed and fixed an additional 2 hr in the same solution at 4C. For electron microscopic studies,
a range of fixative strengths were employed containing 2 or 4 % formaldehyde and 0.5%-2.5% glutaraldehyde. The tissue was postfixed for 2 hr in the same fixative.

After removal of the brain from the skull, coronal or sagital sections through striatum, cerebellum and hippocampus were cut at a thickness of 50-80 um with a
Vibrating Microtome (Leica, model VT 1000E).

As a control, we also labeled cultured bovine aortic endothelial cells, fixed using the same conditions as above, which possess characteristic bundles of actin
filaments called stress fibers. Details about culturing methods are given in Deerinck et al. (1994).

2) Electron microscopic analysis using photooxidation of eosin-phalloidin:

Vibratome sections were washed with 50 mM glycine-PBS containing 0.5% cold water fish gelatin to block nonspecific binding. Following 30 min of washing, the sections
were incubated with agitation in a solution of 0.05% of eosin-phalloidin in 0.5% cold water fish gelatin/50mM glycine-PBS 2 hr. For light microscopy studies, phalloidin
conjugated to rhodamine was also used because of its superior fluorescent quantum yield. As a negative control, the eosin-phalloidin was omitted.

Fluorescent and transmitted light images were recorded using a Zeiss Axiovert inverted microscope with a laser scanning confocal attachment (MRC-1024; Bio-RAD Laboratories,
Cambridge, MA) and a krypton/argon mixed gas laser. Images were collected digitally using either a 40X oil (n.a. =1.3) or 63X (n.a. =1.4) oil objective and transferred to a
graphics program (Adobe Photoshop 5.0).

3) Photooxidation:

After additional washes in sodium cacodylate buffer, tissue sections labeled with eosin-phalloidin were mounted on glass-welled tissue culture dishes
(Mat Tek Corp) pretreated with Cell Tak adhesive (Collaborative Research Inc). Slices were fixed again for 2-5 min with 2% glutaraldehyde in 0.1 M cacodylate buffer, rinsed
in buffer for several minutes, and placed in 50mM glycine and potassium cyanide in cacodylate buffer for an additional 5 min to reduce nonspecific staining. Photooxidation
was performed on the Zeiss Axiovert described above, equipped with a 75W xenon arc light source. Specimens were viewed with a 40X oil objective, n.a. 1.3. Three areas
were chosen for electron microscopic analysis: cerebellar molecular layer, dorsal striatum and hippocampal area CA1. The appropriate areas were located with transmitted
light and the pattern of fluorescent labeling was recorded using the confocal attachment at a low laser power setting. The samples were immersed for ten minutes in a solution
of 2.8 mM DAB in 0.1 M sodium cacodylate at 4C bubbled with pure O_2, final pH 7.4, and then irradiated under conventional epifluorescence using a xenon lamp. The DAB solution
was changed every few minutes while the reaction proceeded. Continuous observations were made during the photooxidation procedure using transmitted light. After 6-8 min.,
a brownish reaction product began to appear in place of the fluorescence. The process was stopped by halting the excitation (Deerinck et al., 1994).

Following photooxidation, tissue sections were rinsed in 0.1M sodium cacodylate several times and incubated for 30 min with 1% osmium tetroxide in 0.1M
sodium cacodylate, pH 7.4. Some sections were fixed for 1 hr in 2.25% glutaraldehyde with 0.2% tannic acid added both in cacodylate buffer. Osmication was done with 0.75 %
OsO_4 in cacodylate buffer, pH 6, for 1 hr on ice. Treatment with tannic acid and osmication at low pH is known to protect

actin filaments from depolymerization during osmium fixation (Pollard and Maupin, 1982). After several washes with ddH20, slices were dehydrated in an ascending ethanol series, infiltrated with Durcopan ACM resin and polymerized for 24 hr at 60C. Thin sections (80-100 nm) and thick sections (0.5-1um) were cut with Reichert Ultracut E using glass knives. Thin sections were examined using a JEOL 100CX electron microscope at 80-100 keV and thick sections were observed using a JEOL JEM-4000EX intermediate voltage microscope (IVEM) at 400 keV. One set of thin sections was poststained with a combination of uranyl acetate and lead citrate, but most were examined without additional counterstain. Stereopairs were generated by tilting the specimen 5 degrees between micrographs.

Image Type -	
SINGLE_TILT_IMAGE_SEQ_ID	5
TILT_INCREMENT	2 degrees
SINGLETILTIMAGESEQ_ID	5
TILT_INCREMENT	2 degrees
RANGE_MAX	60 degrees
RANGE_MIN	-60 degrees

Specimen Description -	
ANATOMICAL_DETAIL	22
ATLAS_COORD	, ,
CELL_TYPE	Purkinje neuron
ORGAN	brain
REGION	cerebellum
STRUCTURE	neuropil
SYSTEM	central nervous system

Electron Microscopy Product -	
EM_PRODUCT_ID	5
ACCELERATING_VOLTAGE	400 KeV
MAGNIFICATION	40000
RECORDING_MEDIUM	film

Raw 2D Image

Raw Low Resolution 2D Image -

Raw 2D Image -	
IMAGE2D_ID	22
IMAGE_FILE_NAME	cere14/cere14_img.jpg
RAW_ANIMATION_FILE	cere14/cere14_img.qt
RAW_DATA_FILE	cere14/cere14_img.tar

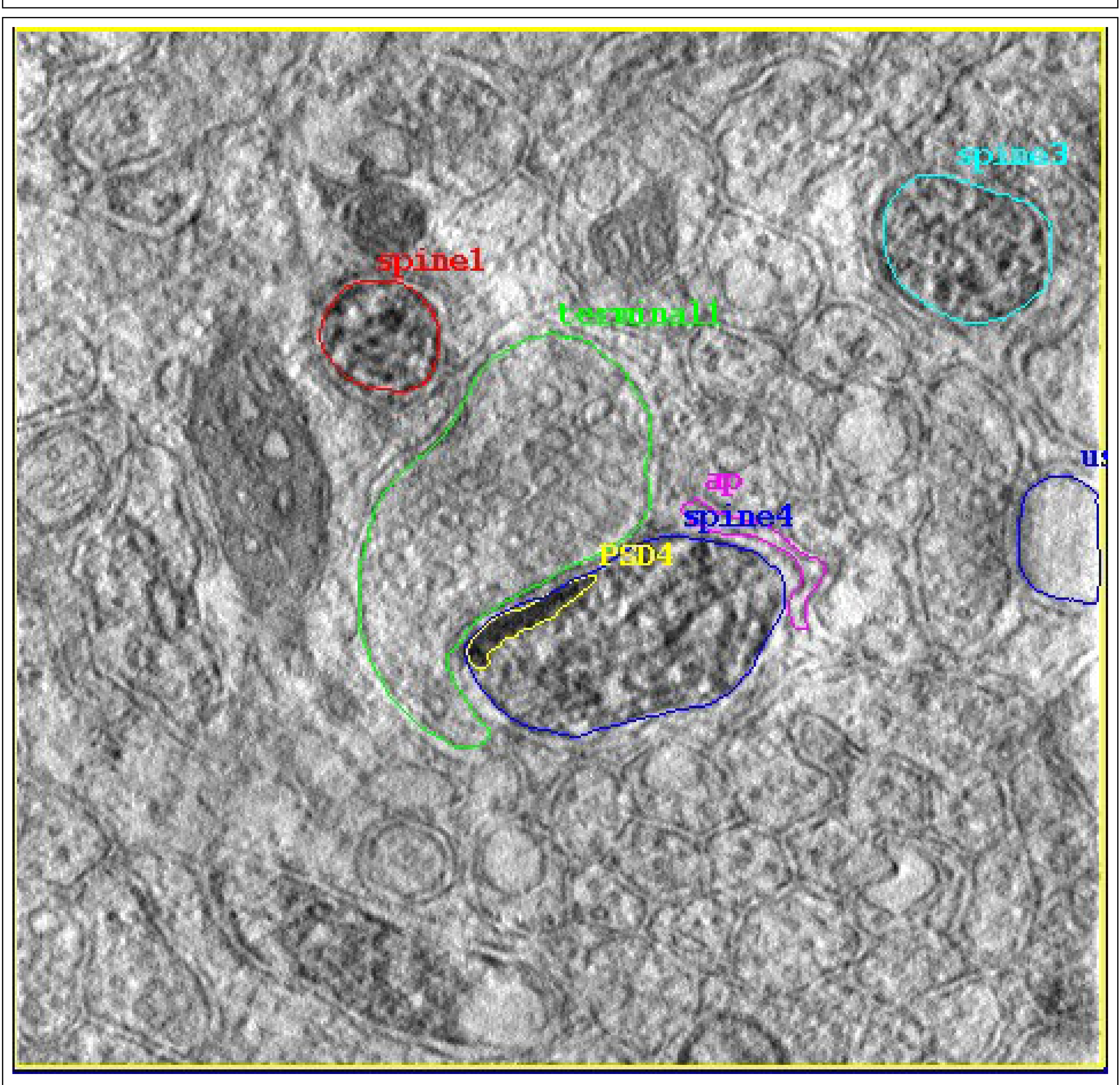
Reconstruction

Reconstruction Image -

Reconstruction -	
RECONSTRUCTION3D_ID	22
ALIGNMENT_PROGRAM	manual
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
RECON_ALGORITHM	R-weighted back projection
RECON_DATE	2000-02-08 00:00:00.0
RECON_DESC	Reconstruction of cerebellar neuropil using phalloidin-photooxidation
RECON_PROGRAM	Suprim
RECON_TYPE	single tilt electron tomography
THUMBNAIL	P1134/cere14_vt.jpg
VOLUME_DIMENSION	836, 798, 166
VOLUME_NAME	cere14/cere14_vol.tar
VOXEL_SCALE	.0012, .0012, .0012
RECONSTRUCTION_IMAGES_ID	22
RECON_IMAGE_DESC	single slice through the middle of a tomographic reconstruction of Purkinje cell spines labeled for F-actin
RECON_FILE_NAME	cere14/cere14_slice69.jpg
VOLUME_THUMBNAIL	P1134/cere14_vt.jpg
ANIMATION_FILE	cere14/cere14_mm_sub.qt
ANIMATION_DESC	Slices through cerebellar molecular layer neuropil showing several examples of dendritic spines labeled for F-actin using phalloidin-photooxidation

Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	84
LABELING_INTENSITY	7.805
LABELING_RANK	low
OBJECT_DESC	axonal terminal
OBJECT_NAME	terminal1
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	84
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	85
ANALYZE_DESC	Labeling intensity determined using Xvoxtrace: average intensity value was computed over the area of the contours comprising the object.Background intensity from similar unlabeled objects was subtracted from each value.
ANALYZE_DESC	Labeling intensity determined using Xvoxtrace: average intensity value was computed over the area of the contours comprising the object.Background intensity from similar unlabeled objects was subtracted from each value.
IS_MANUAL	Y
LABELING_INTENSITY	46.25
LABELING_RANK	high
OBJECT_DESC	labeled dendritic spine
OBJECT_NAME	spine1
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	85
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEG_FILE_NAME	cere14_2.trace
THUMBNAIL	P1134/cere14_st.jpg
SEGMENTED_OBJECT_ID	86
LABELING_INTENSITY	29.9
LABELING_RANK	high
OBJECT_DESC	labeled dendritic spine
OBJECT_NAME	spine2
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	86
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	87
LABELING_INTENSITY	43.95
LABELING_RANK	high

Segmentation -

OBJECT_DESC	labeled dendritic spine
OBJECT_NAME	spine3
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	87
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	88
LABELING_INTENSITY	34.08
LABELING_RANK	high
OBJECT_DESC	labeled dendritic spine
OBJECT_NAME	spine4
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	88
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	89
LABELING_INTENSITY	.395
LABELING_RANK	none
OBJECT_DESC	parallel fibers
OBJECT_NAME	pf
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	89
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	90
LABELING_INTENSITY	39.42
LABELING_RANK	high
OBJECT_DESC	post synaptic density
OBJECT_NAME	PSD4
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	90
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	91
LABELING_INTENSITY	60.14
LABELING_RANK	high
OBJECT_DESC	post synaptic density
OBJECT_NAME	PSD2
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg

Segmentation -

SEGMENTED_OBJECT_ID	91
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	92
LABELING_INTENSITY	38.11
LABELING_RANK	high
OBJECT_DESC	post synaptic density
OBJECT_NAME	PSD1
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	92
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace
SEGMENTED_OBJECT_ID	93
LABELING_INTENSITY	1.745
LABELING_RANK	none
OBJECT_DESC	astrocytic process
OBJECT_NAME	ap
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	cere14/a_cere14.jpg
SEGMENTED_OBJECT_ID	93
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	manual segmentation using Xvoxtrace

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.

DISCLAIMER

THE DATA PROVIDED BY THE CCDB ARE FREELY DISTRIBUTED AND WITHOUT CHARGE. THESE DATA ARE PROVIDED BY THE CCDB "AS IS" AND WITHOUT ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT, TO ANY THIRD PARTY RIGHTS. IN NO EVENT SHALL THE CCDB BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THESE DATA, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE.

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