

Cell Centered Database

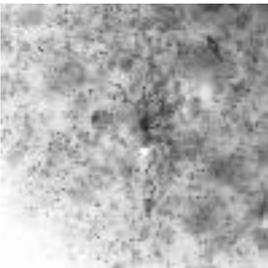
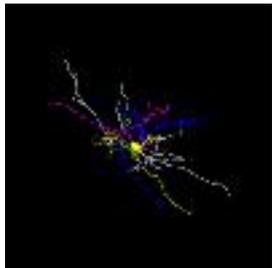
University of California, San Diego

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Microscopy Product #:3379 050803A

For the most updated information, please visit

<http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=3379>

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1207
PROJECT_NAME	Correlative microscopic characterization of dendritic spines in a transgenic mouse model of hyperdopaminergia: The dopamine transporter knockout mouse
PROJECT_DESCRIPTION	Multiscale characterization of DAT KO transgenic mouse
LEADER	Diana Price
FUNDING_AGENCY	NIH
PROJECT_START_DATE	2003-01-01 00:00:00.0
PROJECT_END_DATE	
COLLABORATORS	Aki Laakso, Michele Cyr, Maryann Martone , Naoko Yamada , Andrea Thor , Monica Berlanga
PUBLICATION1	
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	EMT reconstructions of medium spiny neuron dendrites
TITLE	P1207 Experiment 5
EXPERIMENTER	Diana Price, Masako Terada, Andrea Thor
EXPERIMENT_NAME	
EXPERIMENT_DATE	2003-04-22 00:00:00.0

Subject Information -	
GROUP_BY	genetic manipulation
SUBJECT_NAME	wildtype/control
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	Mus Musculus
SPECIES	Mouse
STRAIN	C57BL/129SvJ
AGE	7 months
AGECLASS	Adult
ANIMAL_NAME	
LITTER_ID	
SEX	male
VENDOR	
WEIGHT	34 grams

Tissue -	
ANATOMIC_LOCATION	Neostriatum 050803A
MICROTOME	Vibratome
ORIENTATION	Coronal
THICKNESS	100 um
TISSUE_PROD_STORAGE	P1207 slide box 1
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	3379
IMAGE_BASENAME	050803A
CREATE_DATE	2003-05-08 00:00:00.0
INSTRUMENT	Bio-Rad Radiance 2000
MICROSCOPE_TYPE	Confocal
PLANE_COUNT	62
PRODUCT_TYPE	THROUGH FOCUS SERIES
PURL	
SESSION_NAME	
TELESCIENCE_SRB	P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3379
X_RESOLUTION	.19 um/pixels
Y_RESOLUTION	.19 um/pixels
XSIZE	1024
YSIZE	1024

Protocol:

Experiment #5 DAT KO mouse 04/22/03

Description: Photoconverted dye-filled striatal medium spiny

neurons for EM

Animal Info: ID# wt3 wt4

Weight: 34g 32g

DOB: 9/30/02 9/30/02

Protocol

1.Perfusion (at Duke)

Nembutal; 4% paraformaldehyde + 0.1% gluteraldehyde

2.Sectioned on Vibratome (at NCMIR)

Thickness = 100 microns

Store in 1X PBS in fridge

3.Fill cells with Lucifer yellow

4.Store slices with filled cells in 4% para in fridge

5.Wash 6x with PBS 1X (on ice)

6.When ready to begin photoconversion, turn on the chiller in

confocal room. Set at ~4°C. The refrigerator unit should be set

at TEMP < 45°C. Switch ON. Stage needs around 20 minutes to

come to temperature. Pull unit out into hallway (to avoid

increase in temperature).

6.Place slices in 2% glut/PBS on ice for 15 minutes

0.8 ml 25% gluteraldehyde

2 ml 5x PBS

6.2ml ddH₂O

7.Briefly wash slices in PBS

8.Place slices in PBS/glycine for a few minutes

38 mg glycine

10 ml 1x PBS

9.Follow instructions for Photoconversion of Lucifer Yellow-

filled cells

10. After photoconversion, remove DAB solution and wash slice 3x 10 minutes in generous volumes of PBS on ice. Must remove all DAB before beginning osmification.

Microwaving protocol for osmication, dehydration, and embedding of photoconverted slices

*Prepare Resin mix and let it sit covered and undisturbed until needed (instructions by fume hood in embedding area).

*Rinse slices with a generous amount of cold 1X PBS on ice for ~ 10 min.

*Turn on circulating bath (over 20°C, ~ RT): water bath (left hand side) will fill.

*Insert temperature probe

*Fill other T-beaker with water

*Set temperature to 35°C

*Open new bottle of 100% ethanol and prepare following

dilutions:

90% ethanol

70% ethanol

50% ethanol

*Make up osmium solution under fume hood and chill on ice

*1% osmium tetroxide in PBS on ice.

2.0 ml PBS 5X

then 5.5 2x distilled H₂O

2.5 ml Osmium 4%

*Rinse w/ 2x distilled H₂O ? 3 x 5min

*Warm up microwave for 2 minutes on high

*Label tubes & place in rack on ice

*Fill tubes with osmium solution (w/ meniscus at 0.5)

*Using glass hooks, transfer slices to tubes

*Remove temperature probe & set temp above 50°C.

*Put rack w. tubes in for 40 sec at full power

*Change rear water load in T-beaker

*Change osmium solution on ice and microwave for another 40
seconds at full power

*Rinse samples for 2 minutes in distilled water on benchtop
(at RT)

*Insert petri bath with H₂O under rack

*Dehydration steps (2 x 40 seconds per step; all @ 35°C)

1st

2nd

50% EtOH

70% EtOH

90% EtOH

100% EtOH

100% Acetone

*All of the dehydration steps should be carried out in
microcentrifuge tubes filled with 600 ml of solution.

Temperature probe should be in petri dish and set for 35.

Change water in rear water load when warm to touch.

*Change from water to acetone in petri bath under rack ?

check acetone bath level every 3 minutes

*Infiltration steps (both @ 50°C):

With a 50/50 mixture of resin and acetone:

1 x 15

min

1:1 Resin:acetone

* Check rear water load at

7.5 minutes

Switch to 100% resin for 3 x 10 minutes:

1st

2nd

3rd

100% Resin

*Periodically check rear water

load

*Flat embed samples between mould release slides and place

in embedding oven under vacuum.

Image Type -	
THROUGH_FOCUS_SERIES_ID	6062
ZSTEP	.5um
THROUGH_NOTES	Iris size: 2.0; gain 75.4; offset 0.6

Specimen Description -	
ANATOMICAL_DETAIL	6140
ATLAS	Paxinos and Franklin, 2000
ATLAS_COORD	1.375, -3.825, .5
CELL_ID	050803A
CELL_TYPE	medium spiny neuron
MAP_LOCATION	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/050803e_atlasplate28.jpg
ORGAN	brain
REGION	neostriatum
SYSTEM	central nervous
ANATOMICAL_NOTES	atlas coordinates relative to bregma. Right or left hemisphere was not specified. Cell was registered to coronal plate 27

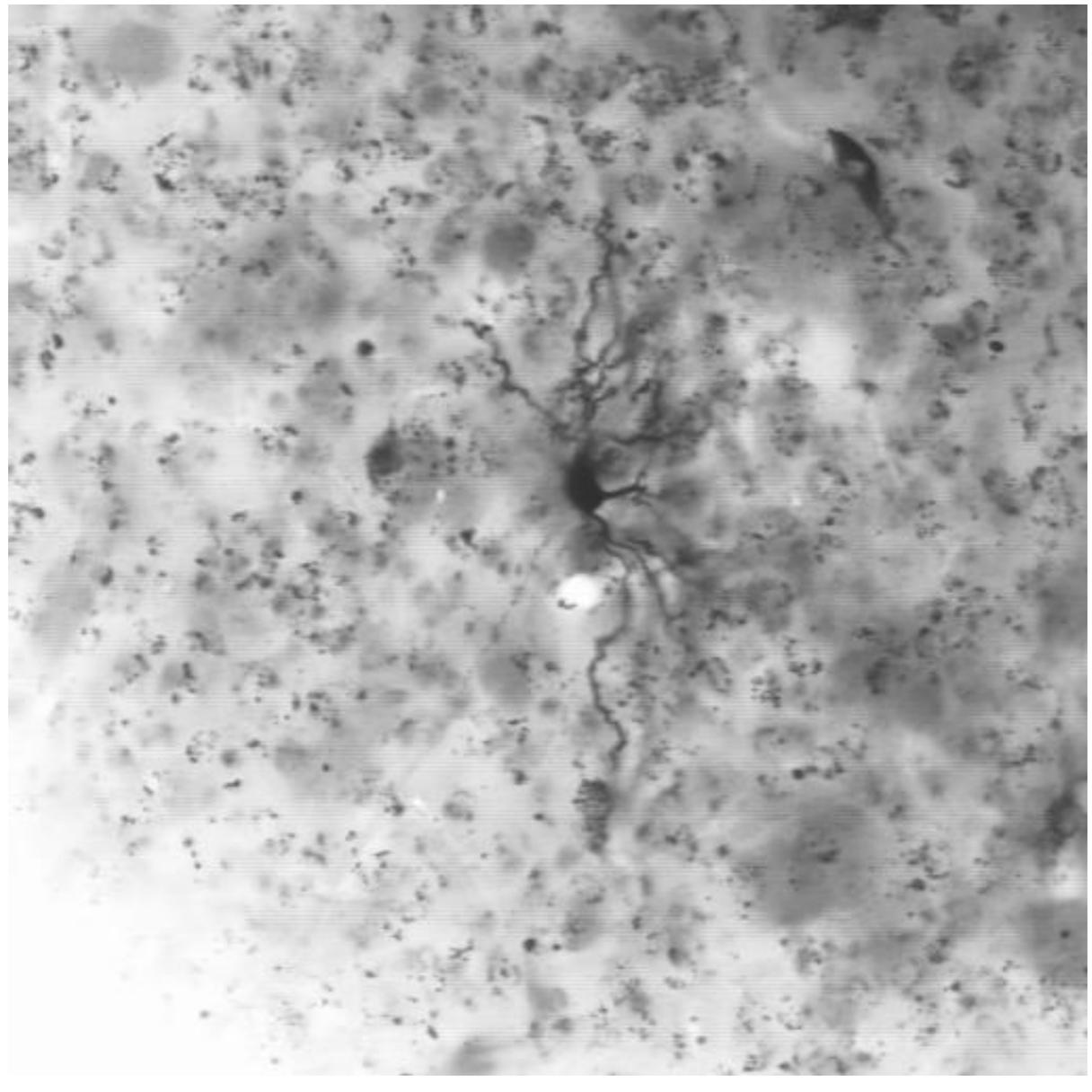
Light Microscopy Product -	
LMPRODUCT_ID	6082

Light Microscopy Product -

COVER_SLIP_THICKNESS	.14 um
IMMERSION_MEDIUM	oil
LENS	Nikon Plan Apo
LENS_MAGNIFICATION	60 X
MOUNTING_MEDIUM	resin
NUMERICAL_APERTURE	1.4
LM_NOTES	mmartone

Raw 2D Image

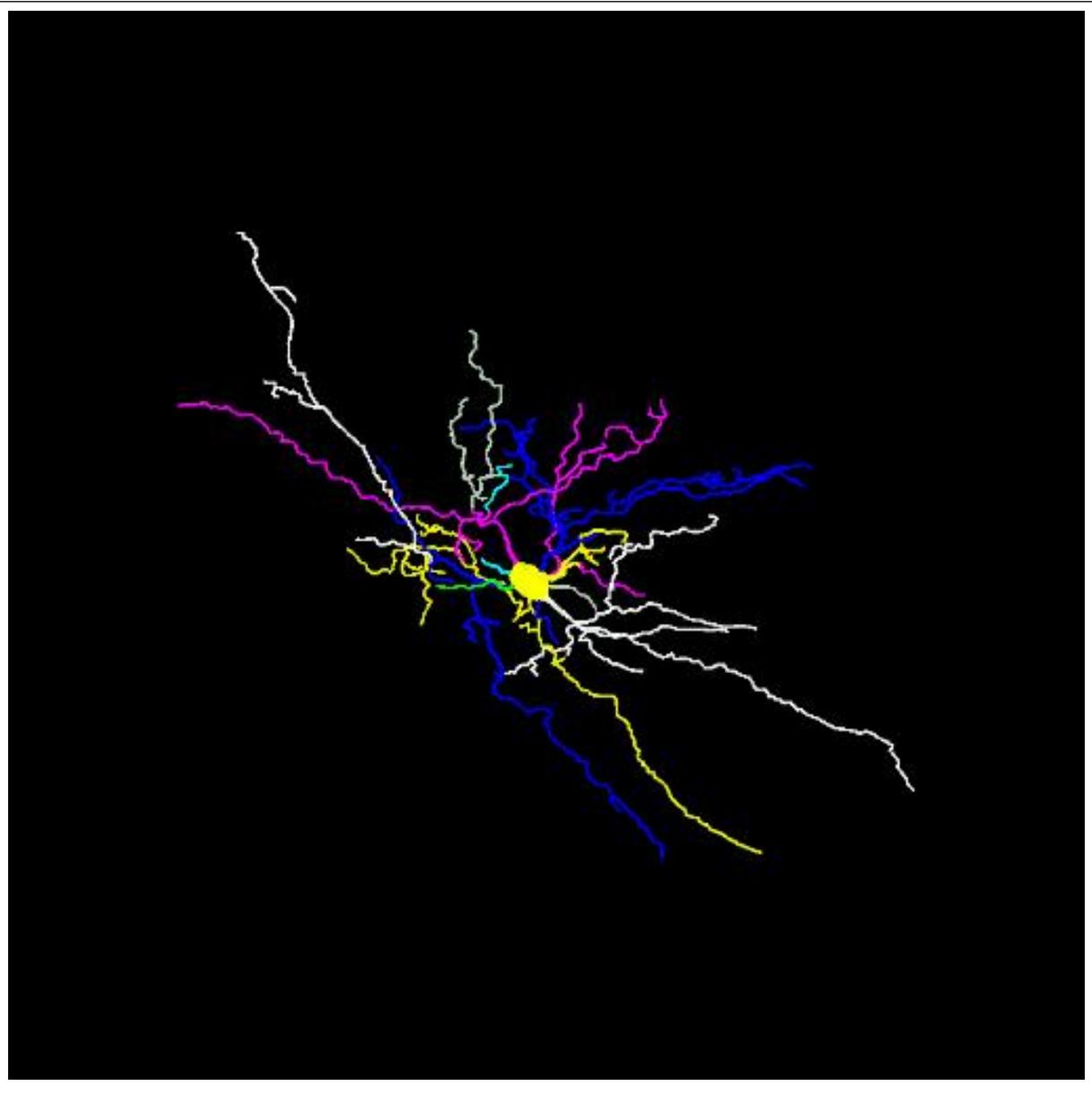
Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	6117
BIT_DEPTH	8 bit
IMAGE_DESC	Zip file containing through focus series in BioRad PIC and tiff format
IMAGE_FILE_FORMAT	Biorad PIC
IMAGE_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/050803e_img.jpg
RAW_ANIMATION_DESC	Animation through the slices of a through focus series of a medium spiny neuron from the neostriatum of a wild type mouse injected with Lucifer Yellow and photoconverted. The flocculent material surrounding the cell likely represent photoconverted mitochondria or just background from the photoconversion procedure.
RAW_ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/050803e_img.avi
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/050803e_img.zip
THUMBNAIL_DESC	Single through focus slice through a medium spiny neuron from the neostriatum of a wild type mouse injected with Lucifer Yellow and photoconverted.
THUMBNAIL_FILE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/050803e_img_thmb.jpg
X_RESOLUTION	.19 um/pixel
Y_RESOLUTION	.19 um/pixel
X_SIZE	1024 pixels
Y_SIZE	1024 pixels

Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	6192
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6191
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6193
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
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SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6194
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
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SEGMENTED_OBJECT_ID	6187
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6188
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6189
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6186
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.zip
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6185
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DENDRITE_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3379/050803a_dendrites.txt
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NEURON_SUMMARY_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3379/050803a_neuronsummary.txt
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/050803e_seg.zip
SPINE_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3379/050803a_spines.txt
THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg_thmb.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6190
DOWNLOADABLE_FILE_DESC	Tar file containing the ascii file output by Neurolucida and a VMRL rendering of the final segmentation, along with the measurement summary files generated by Neuroexplorer for each part of the neuron.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_seg.jpg
SEGMENT_PERSON_NAME	Andrea Thor
SEG_DESC	Manual tracing of dendrites and dendritic spines using Neurolucida. These files were produced in order to situate the correlated electron microscopic volumes into their approximate cellular locations; these are not meant to be highly accurate drawings.
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THUMBNAIL	/telescience/home/CCDB_DATA_USER.portal/P1207/Experiment_19/Subject_49/Tissue_64/Microscopy_3384/0500803e_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 acknowledgement 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