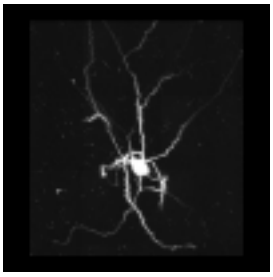
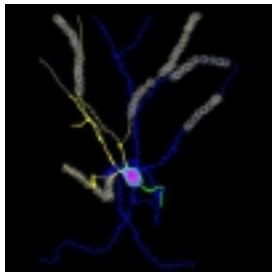
Cell Centered Database

University of California, San Diego

Maryann Martone

Microscopy Product #:1 ACC1

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=1

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P0000
PROJECT_NAME	Mouse BIRN test data
PROJECT_DESCRIPTION	NCMIR test data for Mouse BIRN
LEADER	Maryann Martone
FUNDING_AGENCY	NIH
PROJECT_START_DATE	2001-09-01 00:00:00.0
PROJECT_END_DATE	
COLLABORATORS	Eric Bushong
PUBLICATION1	
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	to obtain multi resolution data for Mouse BIRN
TITLE	Intracellular Injection of Nucleus Accumbens Neuron
EXPERIMENTER	Eric Bushong
EXPERIMENT_NAME	
EXPERIMENT_DATE	2001-12-13 00:00:00.0

Subject Information -	
GROUP_BY	
SUBJECT_NAME	
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	mus musculus
SPECIES	mouse
STRAIN	C57BL/6
AGE	2 months
AGECLASS	adult
ANIMAL_NAME	
LITTER_ID	
SEX	male
VENDOR	
WEIGHT	

Tissue -	
ANATOMIC_LOCATION	neostriatum
MICROTOME	vibratome
ORIENTATION	coronal
THICKNESS	100 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	1
IMAGE_BASENAME	ACC1
CREATE_DATE	2001-12-13 00:00:00.0
INSTRUMENT	BioRad MRC 1024 Confocal
MICROSCOPE_TYPE	multiphoton
PLANE_COUNT	233
PRODUCT_TYPE	Optical section series and mosaic
PURL	NA
SESSION_NAME	
TELESCIENCE_SRB	P0000/Experiment_1/Subject_1/Tissue_1/Microscopy_1
X_RESOLUTION	.119 um
Y_RESOLUTION	.119 um
XSIZE	512
YSIZE	480

Protocol:

Photo-oxidation of Lucifer Yellow Injected Cells

1) Anesthetize rat with ketamine cocktail solution (see below) using 0.4 mL/100 g body weight or Nembutal (0.1mL/100g).

To prepare the ketamine cocktail solution:
ketamine 3.75 ml
acepromazine 0.30 ml
rompun/xylazine 1.90 ml
sterile saline 23.0 ml

Store in an airtight and light protected bottle for up to 3 months.

2) Clear vasculature using Ringer's solution (37¿C):
¿9.9 mL NaCl (79.8 g/L)
¿1 mL KCl (37.5 g/L)
¿1 mL Na2HPO4 (18 g/L)
¿1 mL MgCl2 . 6 H20 (20.0g/L)
¿2.5 mL NaHCO3 (50.0 g/L)
¿Fill to 95.5 mL using ddH2O
¿Warm to 37 ¿C and bubble w/carbogen
¿1 mL CaCl2 . 2 H2O (30.0 g/L)
¿200 mg dextrose
¿2.5 mL heparin
¿1 mL xylocaine

3) Perfuse with fixative for 6-15 minutes, depending on size of animal (6 minutes - mouse; 10 minutes - small rat; 15 minutes - large rat)
using 4% paraformaldehyde in 0.1 M PBS, pH 7.4 (200 mL) at 37¿C:
Heat 100 mL ddH2O to 60¿C
Add 8 g "Prill" paraformaldehyde
Add 4-6 drops 1 N NaOH
Filter with #1 Whatman filter
Add 40 mL 5x PBS
Dilute to final volume of 200 mL

Add 0.1% glutaraldehyde if intending to photoconvert specimens.

4) Extract brain. If it is still soft, place in same fixative as above and allow to post-fix for 0.5-1 hour at 4¿C. Cut into 100-150¿m slices with Vibratome using a slow speed and the lowest frequency which will allow for proper cutting.

5) Store slices in 0.1 M PBS in refrigerator. Slices should be used same day if planning to photoconvert. Given good initial fixation,
slices will be usable for dye-filling for 2-3 days.

6) Visualize tissue using IR-DIC Nomarski imaging. If necessary, counterstain tissue in 5 ?M acridine orange in 0.1 M PBS for 30 seconds.
Microinject cells using 5% Lucifer Yellow-CH (lithium salt) in ddH2O. Use a positive retention current to prevent leakage, if necessary,
and a negative pulsed current of 1-3 nA to inject cells. Allow cells to fill until all processes are equally fluorescent.

7) Place slices containing filled cells into 4% LY at 4¿C for at least 20-30 minutes.

8) Acquire light-level image of cell, using Mat-Tek dish to hold specimen. Mat-Tek dishes should be prepared ahead of time by treating with
polyethyleneimine solution (0.1% aq.) for 30 sec., followed by brief rinse in ddH2O. Allow dish to dry and store in fridge until used.
Can use PBS bubbled with Ar gas to try to reduce fading.

9) Bubble DAB/potassium cyanide solution with O2:
2 mL DAB (10mg/mL)
13 mg potassium cyanide
2.66 mL 5x PBS
8.67 mL dd H2O

9) Fix slice in 2% glutaraldehyde for 15-20 minutes:
0.8 mL 25% glutaraldehyde
2 mL 5x PBS
7.2 mL dd H2O

10) Wash 2-3 times in PBS.

11) Place 100 mM glycine on slice for 1-2 minutes:
38 mg glycine
10 mL PBS

12) Wash with PBS.

13) Incubate slice in DAB/potassium cyanide solution for 8-10 min.

14) Photo-oxidize slice with 75 W xenon lamp. Exchange DAB/potassium cyanide solution every couple of minutes. Allow reaction to proceed until
all fluorescence is gone and brown reaction product is visible (about 10 minutes).

15) Wash 3x 10 minutes in 0.1 M PBS at 4¿C.

Conventional Embedding Procedure

1) Post-fix in 0.5% OsO4 in 0.1 M PBS for 30 minutes at 4¿C.

2) Rinse 3x 2 minutes in ddH2O at 4¿C.

3) Dehydrate as follows: 70% EtOH, 10 minutes; 80% EtOH, 10 minutes; 90% EtOH, 10 minutes; 95% EtOH, 10 minutes; 100% EtOH, 2x 10 minutes;
dry acetone, 2x 10 minutes (2nd time at room temp.)

4) Infiltrate tissue in 50:50 acetone:DurcupanACM for 1 hour (or overnight).

5) 100% resin for 2x 1 hour (2nd time fresh resin).

6) Mount tissue on mould release slides and place in vacuum oven at 60¿C for 2 days.

Microwave-Enhanced Embedding Procedure

1) Osmium fix tissue in 600 ¿L of 1% OsO4 for 2x 40 seconds. Solution should begin at < 10¿C and attain a final temp. of 30-35¿C.
Microcentrifuge tube should not be placed in water bath during irradiation.

2) Rinse samples ddH20 for 2 minutes at room temp.

3) Dehydration:

50% EtOH 2 X 40 sec. 35¿C

70% EtOH 2 X 40 sec. 35¿C
90% EtOH 2 X 40 sec. 35¿C
100% EtOH 2 X 40 sec. 35¿C
Dry Acetone 2 X 40 sec. 35¿C

Dehydration steps performed in water bath. Tubes should be filled with 600 ¿L of solution for each step.

4) Infiltration:

1:1 Resin:acetone 1 X 15 min. 50¿C
100% Resin 3 X 10 min. 50¿C

Fill bath with stock acetone and check level often to ensure that temp. probe is immersed in bath.

5) Polymerization:

Mount tissue on mould release slides and place in vacuum oven at 60¿C for 2 days.

Obtain transmitted light z-series photo-oxidized, if desired, before sectioning.

Photo-oxidation of Lucifer Yellow Injected Cells

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Image Type -	
OPTICAL_SECTION_SERIES	1
CUTTING_PLANE	transverse
OPTICAL_Z_RESOLUTION	.5 um

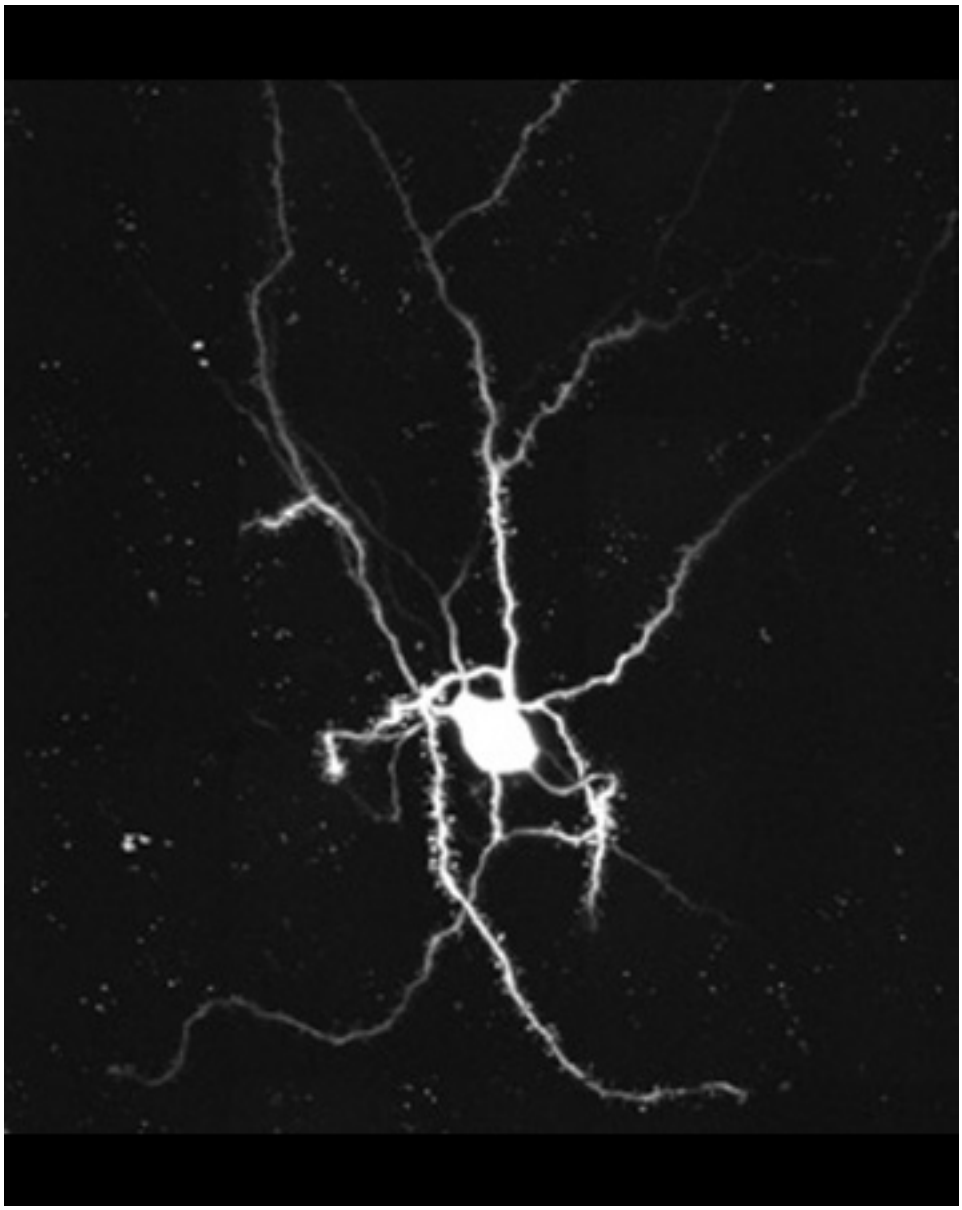
Specimen Description -	

Specimen Description -	
ANATOMICAL_DETAIL	1
ATLAS	Paxinos and Frankliln, 2000
ATLAS_COORD	.75, 4.25, -1.54
CELL_TYPE	medium spiny neuron
ORGAN	brain
REGION	nucleus accumbens
STRUCTURE	dendritic tree
SYSTEM	central nervous system

Light Microscopy Product -	
LMPRODUCT_ID	1
COVER_SLIP_THICKNESS	1 um
IMMERSION_MEDIUM	water
LENS_MAGNIFICATION	60 X
MOUNTING_MEDIUM	water
REFRACTIVE_INDEX	1

Reconstruction

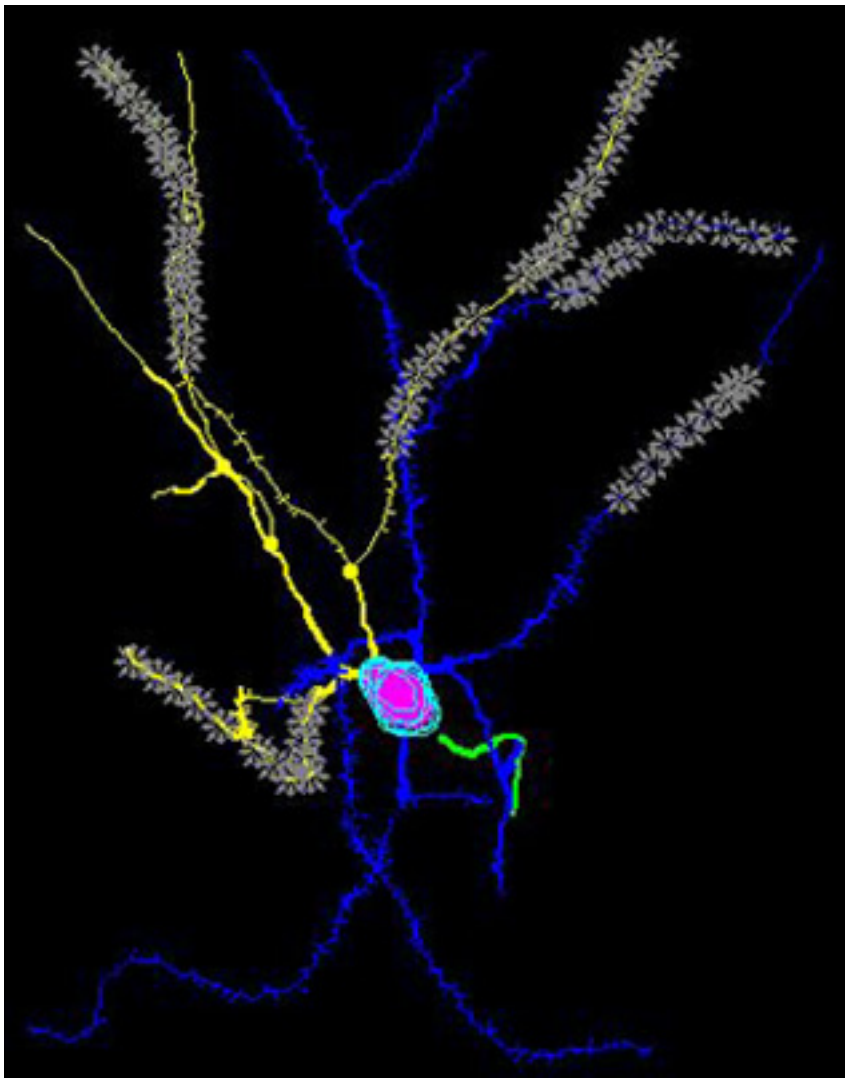
Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	1
CORRELATED_VOLUME_NAME	ACC1_2ma/acc1_2ma_vol.tar
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
DECONVO_PROGRAM	no
IMAGE_MAP_FILE	ACC1/na_montage1.jpg
RECON_DESC	Multi-image tiff file (~570 Mb) containing the optical section series from the tiled mosaic. Note that the name of the file (Proj_EricsMontage.tif) is not the same as the microscopy product basename (acc1).
RECON_TYPE	optical section series/mosaic
THUMBNAIL	P0000/acc1_vt.jpg
VOLUME_DIMENSION	1440, 1782, 233
VOLUME_NAME	ACC1/Proj_EricsMontage.tif
VOXEL_SCALE	.119, .119, .5
RECONSTRUCTION_IMAGES_ID	1
RECON_IMAGE_DESC	Medium spiny neuron from the mouse nucleus accumbens injected with Lucifer Yellow and Alexa 568 and reconstructed from a 3D opcial section series. The Alexa568 channel is shown here. The original data was acquired as a tiled mosaic that was stitched together in X-Y.
RECON_FILE_NAME	ACC1/acc1_thumbnail.jpg
VOLUME_THUMBNAIL	P0000/acc1_vt.jpg
ANIMATION_FILE	ACC1/ACC1_qtmovie.MOV
ANIMATION_DESC	rotation loop of maximum intensity projection of spiny neuron in nucleus accumbens. Some dendrites are incomplete due to the thickness of the section.

Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	1
ANALYZE_DESC	neurolucida
ANALYZE_DESC	neurolucida
DOWNLOADABLE_FILE_DESC	Output of Neurolucida tracing program in ascii format (acc1.spines3c.asc).
IS_MANUAL	y
LABELING_RANK	none
OBJECT_DESC	traced tree
OBJECT_TYPE	tree
SEGMENTED_OBJ_2D_IMAGE	ACC1/acc1_neuro2d.jpg
SEGMENTED_OBJECT_ID	1
SEGMENT_PERSON_NAME	Maryann Martone
SEG_DESC	Segmentation of dendritic tree from medium spiny neuron from the nucleus accumbens of the mouse using Neurolucida. Portions of the dendrite in which the spines were too dim to trace are indicated byt the gray *.
SEG_FILE_NAME	ACC1/acc1.spines3c.asc
THUMBNAIL	P0000/acc1_st.jpg

USER AGREEMENT

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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