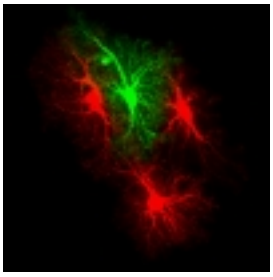
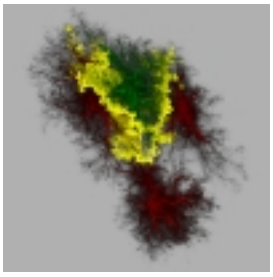
Cell Centered Database

University of California, San Diego

Maryann Martone

Microscopy Product #:28 grp2

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=28

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1114
PROJECT_NAME	Astrocyte Domains
PROJECT_DESCRIPTION	Investigation of astrocyte morphology and the establishment of unique astrocytic domains
LEADER	Eric Bushong
FUNDING_AGENCY	NIH
PROJECT_START_DATE	1999-09-01 00:00:00.0
PROJECT_END_DATE	2002-01-01 00:00:00.0
COLLABORATORS	Maryann Martone and Maryann Martone
PUBLICATION1	Eric A. Bushong; Maryann E. Martone; Ying Z. Jones; and Mark H. Ellisman. Protoplasmic Astrocytes in CA1 Stratum Radiatum Occupy Separate Anatomical Domains. J. Neurosci. 2002 22: 183-192.
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	Extent of overlap of domains of two neighboring astrocytes in CA1
TITLE	Astrocyte overlap using two color injection
EXPERIMENTER	Eric Bushong
EXPERIMENT_NAME	
EXPERIMENT_DATE	2000-01-01 00:00:00.0

Subject Information -	
GROUP_BY	
SUBJECT_NAME	
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	rattus norvegicus
SPECIES	rat
STRAIN	Sprague Dawley
AGE	1 months
AGECLASS	adult
ANIMAL_NAME	
LITTER_ID	
SEX	male
VENDOR	
WEIGHT	

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

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	28
IMAGE_BASENAME	grp2
CREATE_DATE	
INSTRUMENT	Biorad Radiance 2000 Confocal
MICROSCOPE_TYPE	confocal
PLANE_COUNT	65
PRODUCT_TYPE	optical section series
PURL	11756501
SESSION_NAME	
TELESCIENCE_SRB	P1114/Experiment_16/Subject_16/Tissue_21/Microscopy_28
X_RESOLUTION	.14 um
Y_RESOLUTION	.14 um
XSIZE	1024
YSIZE	1024

Protocol:

Intracellular fills of astrocytes with fluorescent dyes in fixed tissue
The method for filling cells in fixed tissue slices was adapted from previously reported protocols (Buhl, 1993; Belichenko and Dahlstr¿m, 1995). Male Sprague-Dawley rats, one month of age, were anesthetized with an overdose of Nembutal (10 mg / 100 g body weight) and perfused transcardially with oxygenated Ringer's solution at 37¿C (0.79%

NaCl, 0.038% KCl, 0.020% MgCl2¿6H2O, 0.018% Na2HPO4, 0.125% NaHCO3, 0.030% CaCl2¿2H2O, 0.20% dextrose, 0.020% xylocaine) for about 30 seconds, followed by 0.1 M phosphate buffered saline, pH 7.4 (PBS) containing 4% paraformaldehyde (37¿C). For electron microscopic studies, 0.1% glutaraldehyde was added to the fixative. The fixative was perfused through the body for 10 minutes, at which point the brain was removed and cut on a vibratome into 100-¿m thick coronal slices. The slices were stored in ice-cold PBS and used within 48 hours. The slices were placed in cold PBS and viewed with an Olympus BX50WI infrared differential interference contrast/epi-fluorescent microscope (Olympus, Melville, NY), using a 60x water immersion objective. Sharp glass micropipettes were pulled on a vertical pipette puller (David Kopf Instruments, Tujunga, CA) using omega-dot capillary tubes (OD 1.00 mm, ID 0.58 mm; resistances ranged between 100 and 400 M?) and back-filled with either 10 mM Alexa Fluor 568 in 200 mM KCl, 10 mM Alexa Fluor 488 in 200 mM KCl (Molecular Probes, Eugene, OR), or 5% aqueous dilithium Lucifer Yellow CH (LY) (Calbiochem, La Jolla, CA). The astrocytes were identified by the distinctive size and shape of their soma. The somata were impaled and the dye was injected into the cells by applying a 0.5-second negative current pulse (1 Hz) until the processes were completely filled. After several cells were filled in a tissue slice, the slice was placed in cold 4% paraformaldehyde/PBS for about 30 minutes. At this point the slices could be coverslipped in Gelvatol (Harlow and Lane, 1988) or processed for immunohistochemical labeling.

Immunohistochemical labeling

Tissue slices were washed in 25 mM Tris buffered saline, 0.8% NaCl, pH 7.4 (TBS) for 30 minutes. Slices were blocked in TBS containing 2% NaCl, 3% normal donkey serum (NDS), 1% cold water fish gelatin (CWFG), 1% bovine serum albumin (BSA), and 0.1% Triton-X100 (TX) for 1 hour at 4¿C. The slices were then incubated for 72 hours (4¿C) with guinea pig polyclonal anti-GFAP antibody (Advanced ImmunoChemical, Long Beach, CA) diluted 1:200 in working buffer (TBS containing 2% NaCl, 0.3% NDS, 0.1% CWFG, 0.1% BSA, and 0.25% TX). The slices were then washed 3 x 10 minutes in working buffer and then placed in working buffer (4¿C) containing 1:100 donkey anti-guinea pig IgG conjugated to Cy5 (Jackson ImmunoResearch, West Grove, PA). After 24 hours, the slices were washed 3 x 10 minutes in TBS and then coverslipped in Gelvatol.

Imaging and analysis of dye-filled astrocytes

The Gelvatol anti-fade mounting media was allowed to set overnight. The filled astrocytes were then visualized using confocal laser scanning microscopy. The imaging was performed on a Biorad Radiance2000 microscope with a 60x oil immersion (NA=1.4) Nikon objective. Z-motor calibration was checked using z-series through 15 ¿m fluorescent latex beads. Proper channel alignment was confirmed using z-series through both latex beads and Purkinje cell spines that had been filled with both Lucifer Yellow and Alexa 568. Image visualization and analysis was performed using the Bitplane software suite (Bitplane AG, Zurich, Switzerland).

Image Type -	
OPTICAL_SECTION_SERIES	20
CUTTING_PLANE	transverse
OPTICAL_SECTION_SERIES_DESC	optical section series of four adjacent cells; 3 injected with Alexa 568 and 1 with Alexa 488
OPTICAL_Z_RESOLUTION	.3 um

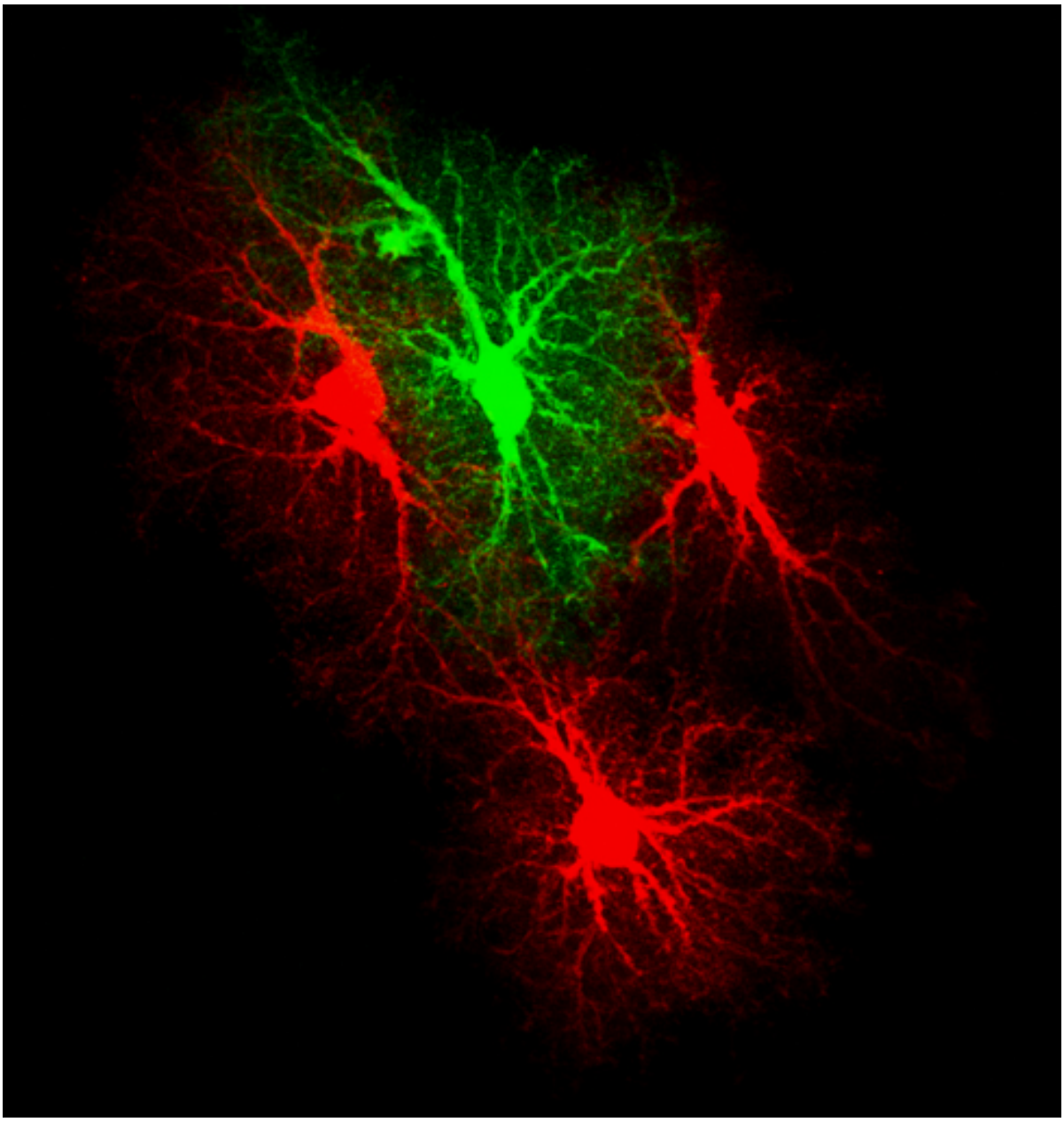
Specimen Description -	
ANATOMICAL_DETAIL	28
ATLAS_COORD	, ,
CELL_TYPE	protoplasmic astrocyte
ORGAN	brain
REGION	hippocampus
SYSTEM	central nervous system

Light Microscopy Product -	

Light Microscopy Product -	
LMPRODUCT_ID	21
COVER_SLIP_THICKNESS	1 um
IMMERSION_MEDIUM	oil
LENS_MAGNIFICATION	60 x
MOUNTING_MEDIUM	gelvatol
NUMERICAL_APERTURE	1.4

Reconstruction

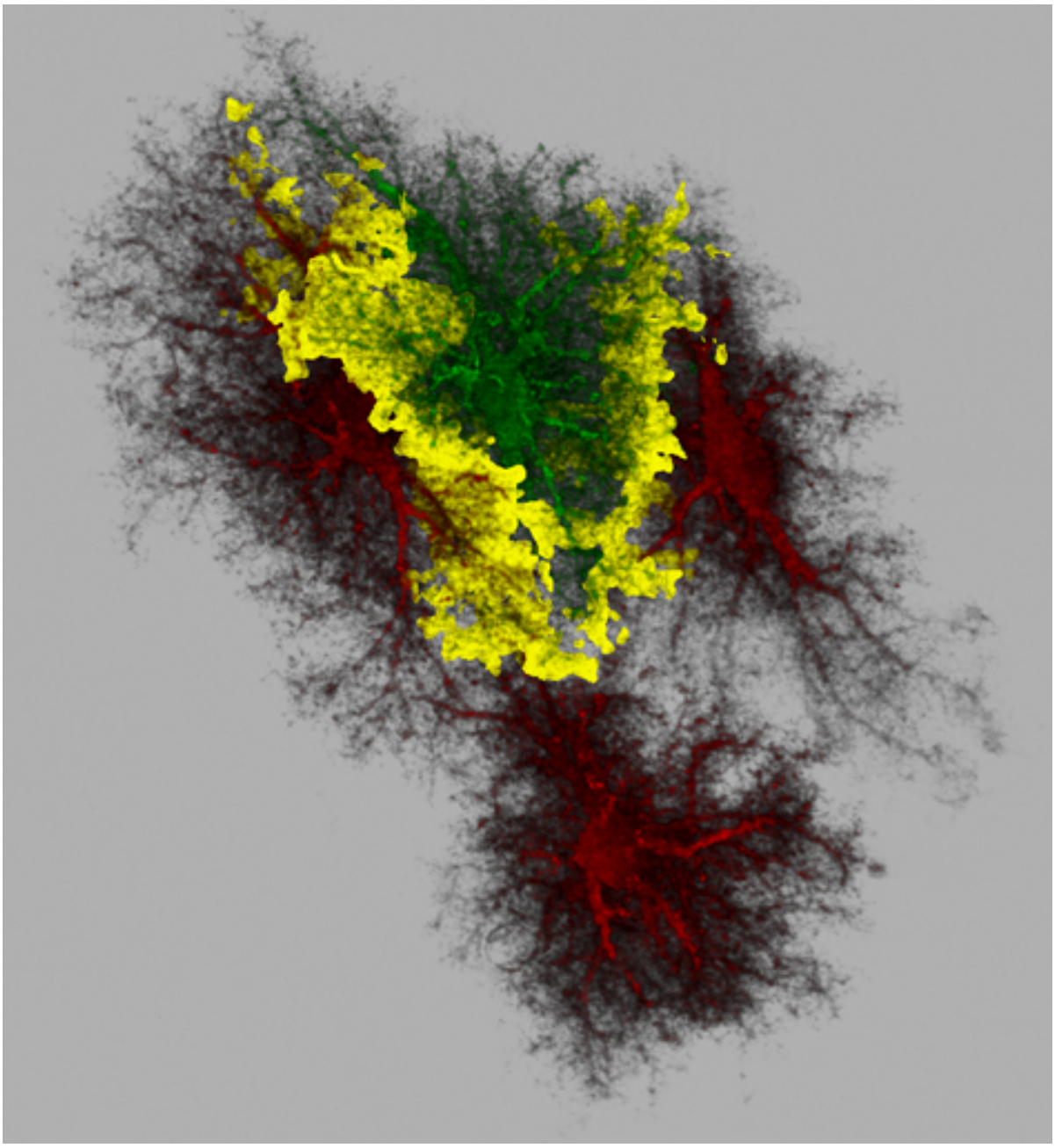
Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	28
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
DECONVO_PROGRAM	no
RECON_DESC	Maximum intensity projection of intracellularly labeled astrocytes in stratum radiatum of CA1.
RECON_PROGRAM	Imaris
RECON_TYPE	optical section series/mosaic
THUMBNAIL	P1114/grp2_vt.jpg
VOLUME_DIMENSION	1024, 1024, 65
VOLUME_NAME	grp2/grp2.tar
VOXEL_SCALE	.14, .14, .3
RECONSTRUCTION_IMAGES_ID	28
RECON_IMAGE_DESC	Maximum intensity projection of filled astrocytes in hippocampal area CA1. See Fig. 7 in Bushong et al. (2002).
RECON_FILE_NAME	grp2/grp2_vol.jpg
VOLUME_THUMBNAIL	P1114/grp2_vt.jpg
ANIMATION_FILE	grp2/grp2-final-seq.avi
ANIMATION_DESC	Slices through optical section series of filled astrocytes showing region of overlap between the processes of adjacent astrocytes (yellow).

Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	178
ANALYZE_DESC	Optical sections through adjacent astrocytes injected with either Alexa488 (green) or Alexa568 (red) in which the area of overlap between adjacent processes has been segmented (yellow)
ANALYZE_DESC	Optical sections through adjacent astrocytes injected with either Alexa488 (green) or Alexa568 (red) in which the area of overlap between adjacent processes has been segmented (yellow)
DOWNLOADABLE_FILE_DESC	Tar file contains individual channel volumes after Gaussian deblurring and the region of colocalization (coloc) as a separate volume file.
IS_MANUAL	N
OBJECT_DESC	colocalization
OBJECT_NAME	coloc
OBJECT_TYPE	volume
SEGMENTED_OBJ_2D_IMAGE	grp2/grp2_seg.jpg
SEGMENTED_OBJECT_ID	178
SEGMENT_PERSON_NAME	Eric Bushong
SEG_FILE_NAME	grp2.tar
THUMBNAIL	P1114/grp2_st.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