Cell Centered Database
University of California, San Diego
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Microscopy Product #:3722 3sl4as4

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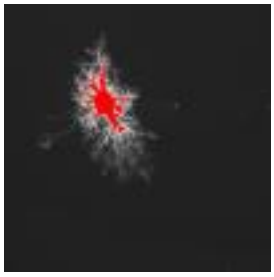
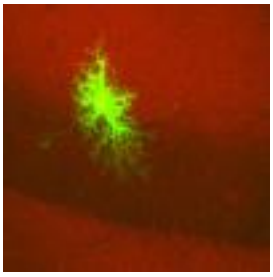
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=3722

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1231
PROJECT_NAME	Laminar Boundaries
PROJECT_DESCRIPTION	Relationship between astrocyte distribution & morpholgy and laminar boundaries in the dentate gyrus
LEADER	Eric Bushong
FUNDING_AGENCY	National Institutes of Health
PROJECT_START_DATE	2000-03-01 00:00:00.0
PROJECT_END_DATE	2003-07-23 00:00:00.0
COLLABORATORS	Maryann Martone, Mark Ellisman
PUBLICATION1	Bushong EA, Martone ME, Ellisman MH. Examination of the relationship between astrocyte morphology and laminar boundaries in the molecular layer of adult dentate gyrus. J Comp Neurol. 2003 Jul 21;462(2):241-51. PMID: 12794746
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	To investigate the relationship of filled astrocytes to laminar boundaries in the dentate gyrus revealed by Epha4 immunostaining
TITLE	Exp3
EXPERIMENTER	Eric Bushong
EXPERIMENT_NAME	
EXPERIMENT_DATE	

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

Tissue -	
ANATOMIC_LOCATION	hippocampus
MICROTOME	Vibratome
ORIENTATION	coronal
THICKNESS	75 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	3722
IMAGE_BASENAME	3sl4as4
CREATE_DATE	
INSTRUMENT	BioRad 1024 MRC Confocal
MICROSCOPE_TYPE	LASER SCANNING CONFOCAL
PLANE_COUNT	106
PRODUCT_TYPE	OPTICAL SECTION
PURL	
SESSION_NAME	
TELESCIENCE_SRB	P1231/Experiment_3410/Subject_115/Tissue_194/Microscopy_3722
X_RESOLUTION	.16 um/pixels
Y_RESOLUTION	.16 um/pixels
XSIZE	1024
YSIZE	1024

Protocol:

Materials

The rabbit anti-EphA4 antibody recognizing the 11 carboxy-terminal amino acids of chicken EphA4 was generously provided by Dr. Elena Pasquale (The Burnham Institute, La Jolla, CA). The production and specificity of the antibody were previously

described (Soans et al., [1994]). The monoclonal anti-S100 antibody was purchased from Sigma (St. Louis, MO). The rat anti-N-CAM monoclonal antibody (isoclone 12F11) was obtained from BD PharMingen (San Diego, CA). Fluorescein isothiocyante (FITC)-conjugated donkey anti-rabbit, Cy5-conjugated donkey anti-mouse, and Cy5-conjugated goat anti-rabbit antibodies were purchased from Jackson ImmunoResearch (West Grove, PA). AlexaFluor 568 hydrazide and AlexaFluor 568- and 488-conjugated goat anti-rat and anti-mouse secondary antibodies (highly cross-absorbed) were obtained from Molecular Probes (Eugene, OR). Dilithium salt of Lucifer Yellow CH (LY) was purchased from Calbiochem (La Jolla, CA).

Intracellular labeling of astrocytes with fluorescent dyes

Intracellular injection of astrocytes in lightly fixed tissue slices was performed as previously described, with some modifications (Buhl et al., [1990]). Male Sprague-Dawley rats, 1 month old, were deeply anesthetized with Nembutal (10 mg/100 g body weight). The animals were transcardially perfused with oxygenated Ringer's solution at 37¿C (0.79% NaCl, 0.038% KCl, 0.02% MgCl2 ¿ 6H2O, 0.018% Na2HPO4, 0.125% NaHCO3, 0.03% CaCl2 ¿ 2H2O, 0.2% dextrose, 0.02% xylocaine), followed by 4% paraformaldehyde (PFA) in PBS (pH 7.4, 37¿C) for 8-10 minutes. The brain was placed in ice-cold PBS and cut into coronal slices with a vibratome at a thickness of 100 m. The slices were stored in PBS at 4¿C until used.

The slices were placed under a 60¿ water objective (NA 1.4) and observed with an Olympus BX50WI microscope using infrared-DIC optics (Olympus, Melville, NY). Astrocytes in the upper blade of the dentate gyrus were identified by the shape and size of their somata. Glass micropipettes (OD 1.00 mm, ID 0.58 mm; resistances 100-400 M) were pulled on a vertical puller (David Kopf Instruments, Tujunga, CA) and backfilled with either 5% aqueous LY or 10 mM AlexaFluor 568 in 200 mM KCl. Astrocytes were impaled and iontophoretically injected with dye using 1-second pulses of negative current (0.5 Hz) for 1-2 minutes. After several cells were filled, the slices were placed in ice-cold 4% PFA for at least 1 hour. The slices were then ready to be immunolabeled.

Immunohistochemistry

For S100 double-labeling with EphA4 or N-CAM, a 1-month-old male Sprague-Dawley rat was perfused as described above, except that the 4% PFA was perfused for 20 minutes. Vibratome slices were cut coronally at a thickness of 75 m. These slices and slices containing dye-filled astrocytes were immunolabeled as described below, with all steps performed at 4¿C.

Slices were washed three times for 10 minutes each in PBS. The slices were incubated for 1 hour in blocking solution (PBS containing 3% normal goat serum, 1% cold water fish gelatin, 0.25% Triton X-100). Slices containing LY-filled astrocytes were then placed in working buffer (WB; PBS containing 0.3% normal goat serum, 0.1% cold water fish gelatin, 0.125% Triton X-100) containing either 7 g/ml EphA4 or 1:200 N-CAM antibody for 48 hours. Slices used in the somata distribution experiment were placed in WB containing 7 g/ml EphA4 or 1:200 N-CAM and 1:200 anti-S100. The slices were washed three times in WB for 10 minutes each and then placed in WB containing secondary antibodies at a concentration of 1:100 for 24 hours. For studying astrocyte morphology near boundaries, astrocytes near the EphA4 boundary were filled with LY, and astrocytes near the N-CAM boundary were filled with AlexaFluor 568. EphA4 was subsequently detected using goat anti-rabbit Cy5, and N-CAM was detected using goat anti-rat AlexaFluor 488. In S100-labeled slices, N-CAM was detected with AlexaFluor488, EphA4 was detected with FITC, and S100 was labeled with either Alexa568 or Cy5, respectively. Slices were washed in PBS three times for 10 minutes each. Slices were coverslipped using Gelvatol (Harlow and Lane, [1988]) and allowed to set overnight at room temperature before they were examined. Donkey serum was used throughout the procedure for double-labeled specimens.

Image acquisition and analysis

Specimens were examined using a Radiance2000 laser scanning confocal system (Bio-Rad, Hercules, CA) attached to a Nikon E600FN microscope (Kanagawa, Japan). A 60¿ oil immersion (NA 1.4) objective was used to image LY-filled astrocytes, and a 40¿ oil immersion (NA 1.3) objective was used to image S100 double-labeled slices.

Image visualization and analysis were performed using the program Imaris 2.7 (Bitplane, Zurich, Switzerland). Baseline subtraction and linear contrast stretch functions were performed on volumes to enhance contrast. Final images were prepared using Adobe Photoshop 7.0 (San Jose, CA). Images of astrocytes near boundaries were constructed by combining an average intensity projection of either EphA4 or N-CAM labeling with a maximum intensity projection of the LY- or AlexaFluor 568-filled astrocyte.

Histograms and graphs were generated using KaleidoGraph (Synergy Software, Reading, PA). All results are provided as mean ¿ SEM. Linear weighted sum (LWS) equals P ¿ (count)dp. The degree of polarization (P) was calculated by measuring the maximum extent of processes from the center of the soma toward the pia and toward the stratum granulosum and then dividing the larger value by the smaller. Astrocytes with longer pia-directed processes were given a positive value, and astrocytes with longer stratum granulosum-directed processes were given a negative value. Astrocyte spatial arrangement was tested for nonuniform distribution by means of a bootstrap procedure (Romano, [1989]). Briefly, the calculated Cram¿r-von Mises (CvM) goodness-of-fit value for the empirical data was compared with the CvM value obtained from 10,000 pseudo-samples (each with a sample size equal to tested dataset), each randomly generated by drawing from the null (uniform) distribution. The resulting P value equals the number of pseudo-samples having CvM values greater than the CvM value of the actual sample, divided by 10,000.

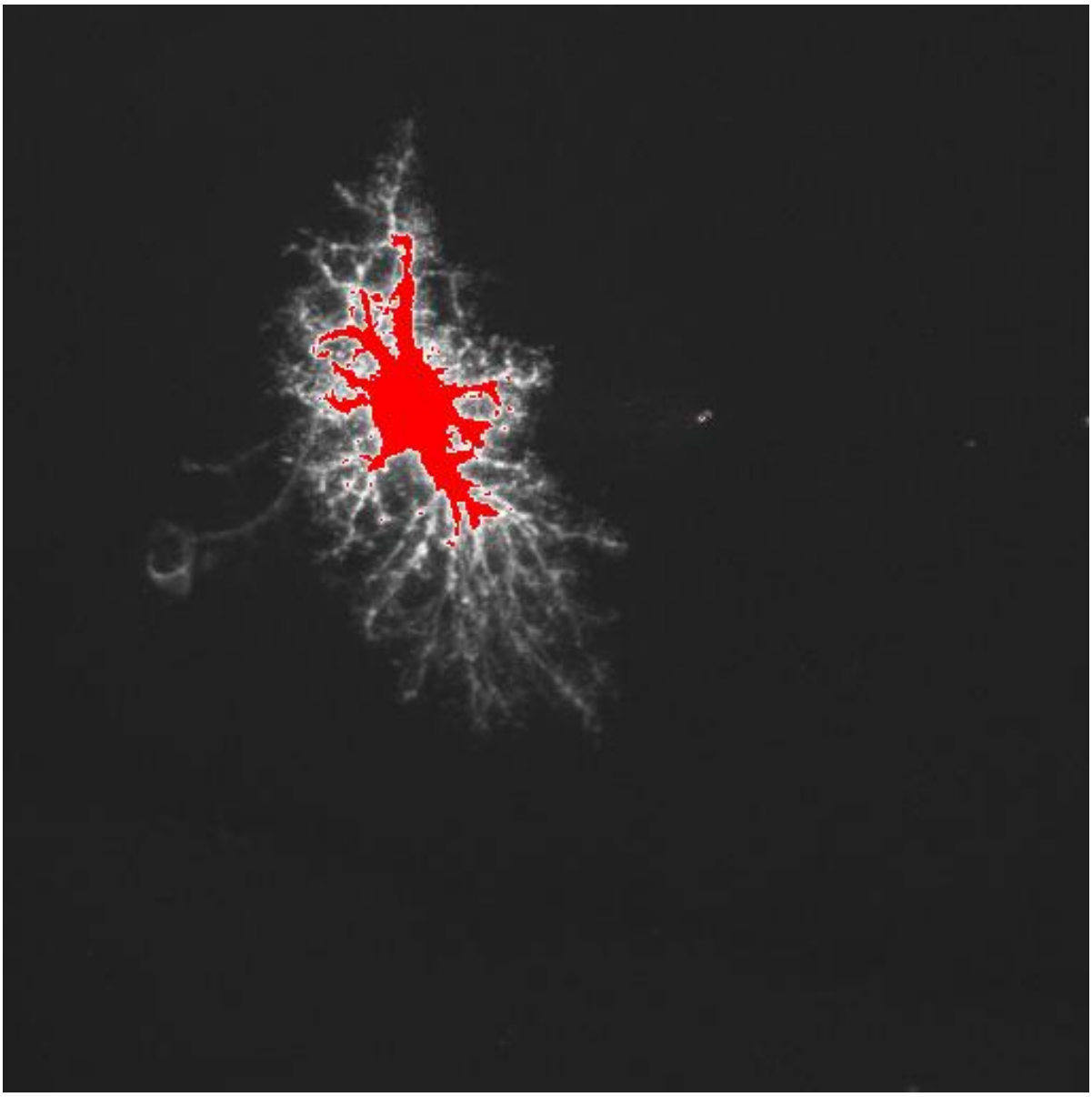
Image Type -	
OPTICAL_SECTION_SERIES	6086
CUTTING_PLANE	transverse
OPTICAL_Z_RESOLUTION	.28 um

Specimen Description -	
ANATOMICAL_DETAIL	15070
ATLAS_COORD	, ,
CELL_TYPE	protoplasmic astrocyte
ORGAN	brain
REGION	dentate gyrus
SYSTEM	central nervous system

Light Microscopy Product -	
LMPRODUCT_ID	6138
IMMERSION_MEDIUM	oil
LENS_MAGNIFICATION	63 X
MOUNTING_MEDIUM	gelvatol
NUMERICAL_APERTURE	1.4
LM_NOTES	null

Raw 2D Image

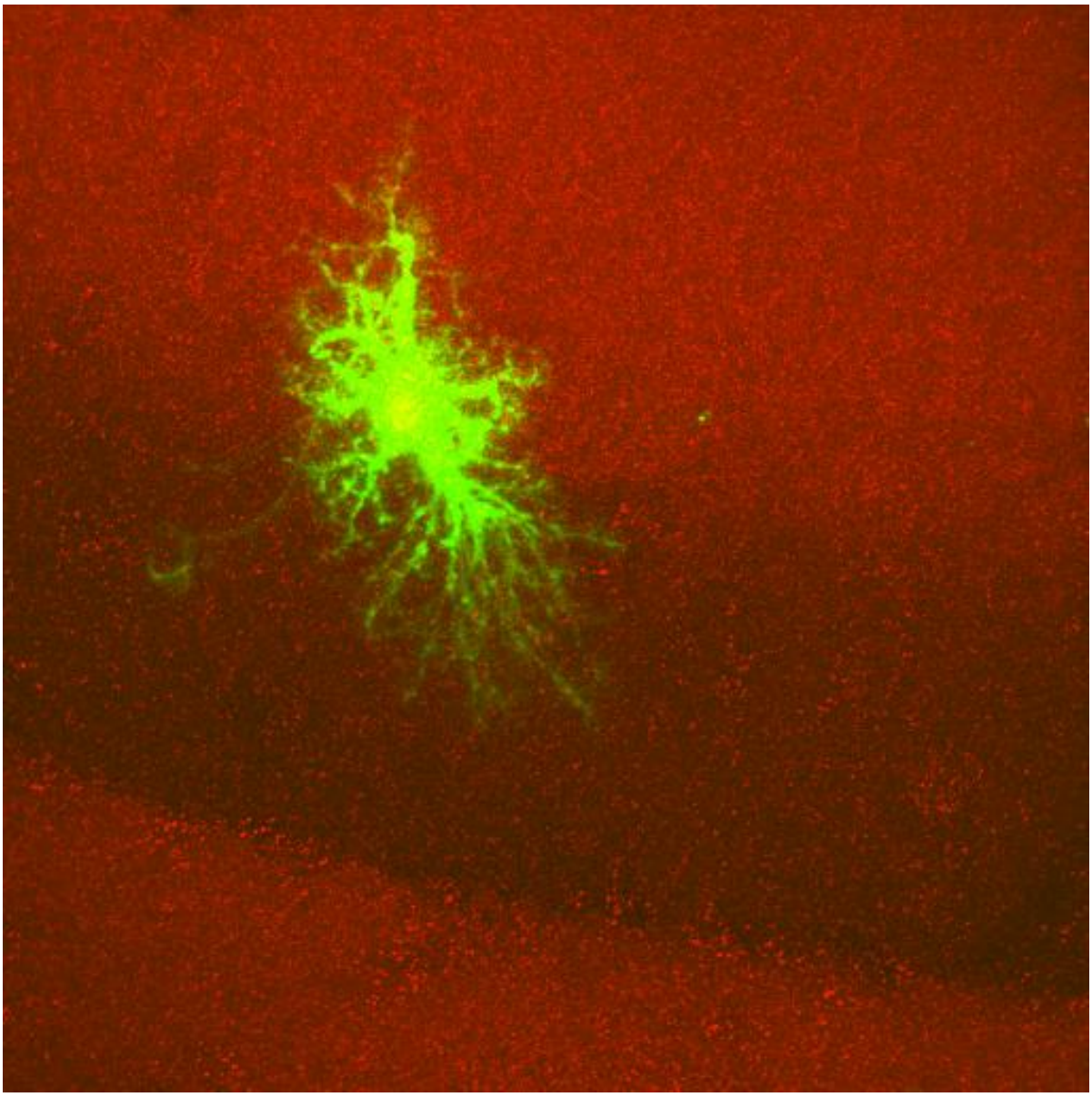
Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	6205
BIT_DEPTH	8 bit
DIGITIZING_PLATFORM	BioRad Radiance2000 confocal
IMAGE_DESC	Zip file containing original optical section series through an intracellularly injected protoplasmic astrocyte (3s14as4_ly.pic) in dentate gyrus immunolabeled for EphA4 (3s14as4_eph.pic). Each label is in a separate file. The merged file is available for download under "Reconstruction."
IMAGE_FILE_FORMAT	BioRad PIC
IMAGE_FILE_NAME	/usr/local/tomcat5.0.28/webapps/FileUploadTool/temp_file_upload/3sl4as4_5122D.jpg
RAW_ANIMATION_DESC	Animation through optical section series of a filled astrocyte in the molecular layer of the rat dentate gyrus imaged using confocal microscopy. Tissue was immunolabeled for EphA4 (see reconstruction animation for merged dataset).
RAW_ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1231/Experiment_3410/Subject_115/Tissue_194/Microscopy_3722/3sl4as4-ly.avi
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1231/Experiment_3410/Subject_115/Tissue_194/Microscopy_3722/3sl4as4_2D.zip
THUMBNAIL_DESC	Projection of an optical section series of a filled astrocyte in the rat dentate gyrus imaged using confocal microscopy. Tissue was immunolabeled for EphA4 (see reconstruction image for merged image).
THUMBNAIL_FILE	/usr/local/tomcat5.0.28/webapps/FileUploadTool/temp_file_upload/3sl4as4_5122D_thmb.jpg
X_RESOLUTION	.16 um/pixel
Y_RESOLUTION	.16 um/pixel
X_SIZE	1024 pixels
Y_SIZE	1024 pixels

Reconstruction

Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	6175
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
RECON_DESC	Zip file containing the merged channel file in tiff format. 3sl4as4_merger.tif.zip
RECON_TYPE	optical section series
VOLUME_DIMENSION	1024, 1024, 106
VOLUME_NAME	/telescience/home/CCDB_DATA_USER.portal/P1231/Experiment_3410/Subject_115/Tissue_194/Microscopy_3722/3sl4as4_merger.tif.zip
VOXEL_SCALE	.16, .16, .28
RECONSTRUCTION_IMAGES_ID	6175
RECON_IMAGE_DESC	Projection through merged optical section series of a filled astrocyte (green) in the molecular layer of the dentate gyrus, stained for Epha4 (red), showing the relationship between astrocyte processes and laminar boundaries revealed by EphA4 staining.
RECON_FILE_NAME	/usr/local/tomcat5.0.28/webapps/FileUploadTool/temp_file_upload/3sl4as4_512R.jpg
VOLUME_THUMBNAIL	/usr/local/tomcat5.0.28/webapps/FileUploadTool/temp_file_upload/3sl4as4_512R_thmb.jpg
ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1231/Experiment_3410/Subject_115/Tissue_194/Microscopy_3722/3sl4as4_merger.avi
ANIMATION_FILE_FORMAT	AVI
ANIMATION_DESC	Animation through the optical sections of a confocal data set showing the relationship of a filled astrocyte (green) to laminar boundaries in the dentate gyrus revealed by EphA4 immunolabeling. the animation has been downsampled from the original data for ease of display.

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