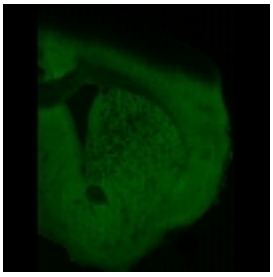
Cell Centered Database

University of California, San Diego

Maryann Martone

Microscopy Product #:30 asyn6

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=30

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1187
PROJECT_NAME	Correlated Imaging Approaches and Multiscale Databases for Research in Parkinson's Disease
PROJECT_DESCRIPTION	characterization of a mouse model of human alpha synuclein overexpressor
LEADER	Diana Price
FUNDING_AGENCY	The Branfman Family Foundation
PROJECT_START_DATE	2002-09-01 00:00:00.0
PROJECT_END_DATE	2003-06-30 00:00:00.0
COLLABORATORS	M.H. Ellisman; M. Martone; G.A. Johnson; E. Masliah
PUBLICATION1	Price DL;Martone ME; Masliah MH; Ellisman MH (2003) High-resolution Large-Scale 3-D Mapping Studies of Alpha-Synuclein Immunoreactivity in Transgenic Mice Overexpressing Human Alpha-Synuclein. Society for Neuroscience Abstract.
PUBLICATION2	Price DL, Chow SK, MacLean NAB, Hakozaki H, Peltier S, Martone ME, Ellisman MH (2006) High-Resolution Large-Scale Mosaic Imaging using Multiphoton Microscopy to Characterize Transgenic Mouse Models of Human Neurological Disorders. Neuroinformatics. 2006;4(1):65-80.
PUBLICATION3	

Experiment Information -	
PURPOSE	to determine the distribution of alpha-synuclein immunolabeling in wildtype tissue
TITLE	Alpha-synuclein immunolabeling for large-scale mapping study
EXPERIMENTER	Diana Price

Experiment Information -	
EXPERIMENT_NAME	
EXPERIMENT_DATE	2003-03-07 00:00:00.0

Subject Information -	
GROUP_BY	genetic manipulation
SUBJECT_NAME	control
FIXATION_METHOD_ID	2
SCIENTIFIC_NAME	mus musculus
SPECIES	mouse
STRAIN	Unspecified
AGE	2 days
AGECLASS	adult
ANIMAL_NAME	
LITTER_ID	
SEX	female
VENDOR	
WEIGHT	25 grams

Tissue -	
ANATOMIC_LOCATION	large scale at level of striatum
MICROTOME	vibratome
ORIENTATION	coronal
THICKNESS	80 um
TISSUE_PROD_STORAGE	p1187 #1
EXTERNAL_FILE_NAME	1187E3_immuno_exp8.doc
TISSUE_GROUP_TYPE	anterior/posterior region

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	30
IMAGE_BASENAME	asyn6
CREATE_DATE	2003-03-25 00:00:00.0
INSTRUMENT	BioRad RTS 2000MP Multiphoton
MICROSCOPE_TYPE	multiphoton
PLANE_COUNT	
PRODUCT_TYPE	optical section series/mosaic
PURL	NA
SESSION_NAME	
TELESCIENCE_SRB	P1187/Experiment_18/Subject_18/Tissue_23/Microscopy_30
X_RESOLUTION	.36 um
Y_RESOLUTION	.36 um
XSIZE	512
YSIZE	480

Protocol:

P1187 Exp. 8 Confocal Study: Branfman Project 3/07/03

Description: alpha-synuclein immunolabeling for large-scale mapping study (non-tg)

Animals: 326 (f) non-transgenic from D-line (see subject log for details)

Protocol

1. Perfusion
Nembutal; 4% paraformaldehyde + 0.1% gluteraldehyde
1 hr. postfix in 4% para

2. Sectioned on Vibratome
Thickness = 80 microns
3 blocks at 2 mm each from anterior (A, B, C) + cerebellum
Left hemisphere marked

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

4. Make up blocking buffer
PBS w/o NaCl = buffer used
Total amount needed = 330 mls

Ingredient Amount
0.8 PBS 66 ml 5X PBS + 242 ml 2x distilled H20
3% NGS (24 , 7) 9.6 ml (~10 vials at 1ml each)
1% fish gel 3.3 ml
0.1% Triton X-100 0.332 ml
1% BSA 3.3 g

5. Block slices (1 hr) in blocking buffer

Ingredient Amount
0.8 PBS 6.6 ml 5X PBS + 24.2 ml 2x distilled H20
3% NGS (24 , 7) 0.96 ml
1% fish gel 0.33 ml
0.1% Triton X-100 0.0332 ml
1% BSA 0.33 g

6. Make up working buffer

Use blocking buffer to dilute to working buffer

Ingredient Amount
Blocking buffer 20 ml
0.1% Triton 0.2 ml
1X PBS 180 ml

7. Wash 1X5 minutes with working buffer

8. Dilute primary Abs in working buffer

anti-alpha-SYN; Host = Rabbit 1:500

9. Place on shaker in cold room labeled & covered with aluminum foil overnight

10. Wash 6x with working buffer

11. Prepare secondary Abs (for confocal immunolabeling)

goat ? rabbit - AF 568 @ 1:50

12. Let sit on cold room shaker covered with foil for 48 hrs

13. Wash 3x with 1X PBS 0.8

14. Prepare nuclear stain
* Final solution = equal parts 2x PBS + 1:100 Hoescht 33342 in ddH2O
* Prepare each separately.
* Once added together, you should not observe any precipitation.
* If precipitation is observed.... Do not use the solution!

15. 30 min staining with nuclear stain

16. Wash 3x with 1X PBS 0.8

17. Sections mounted on slides and coverslipped using Gelvatol

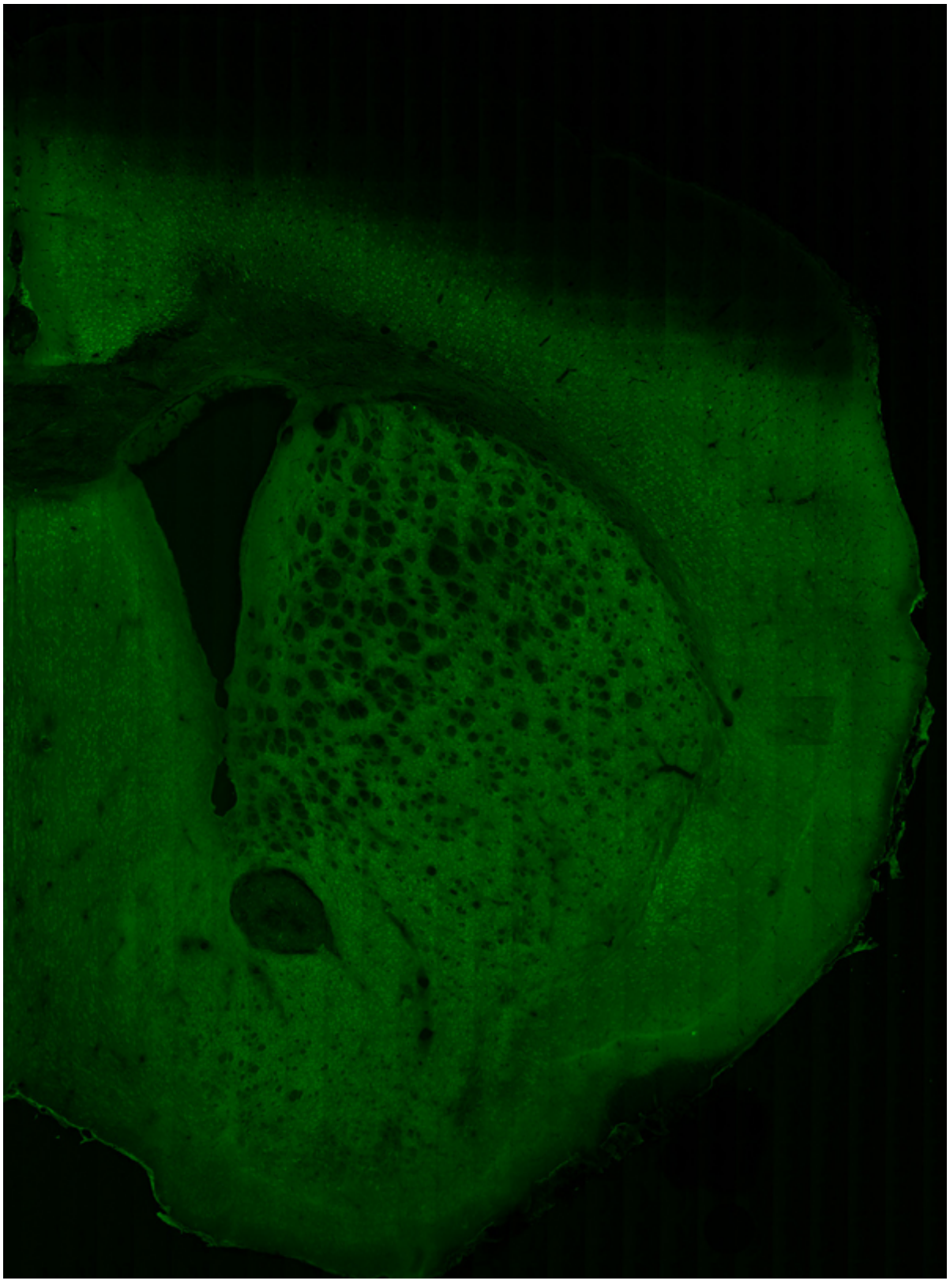
Image Type -	
OPTICAL_SECTION_SERIES	22
CUTTING_PLANE	transverse
OPTICAL_Z_RESOLUTION	2.5 um

Specimen Description -	
ANATOMICAL_DETAIL	30
ATLAS	Paxinos and Franklin
ATLAS_COORD	, , .5
CELL_TYPE	unspecified
MAP_LOCATION	asyn6/P1187_27.jpg
ORGAN	brain
REGION	neostriatum
SYSTEM	central nervous system

Light Microscopy Product -	
LMPRODUCT_ID	23
COVER_SLIP_THICKNESS	1 um
IMMERSION_MEDIUM	oil
LENS	Nikon Plan Fluor
LENS_MAGNIFICATION	40 x
MOUNTING_MEDIUM	gelvatol
NUMERICAL_APERTURE	1.3
REFRACTIVE_INDEX	1

Reconstruction

Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	30
ALIGNMENT_METHOD	automatic
ALIGNMENT_PROGRAM	IMOD
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
RECON_DESC	Manual Alignment
RECON_PROGRAM	IMOD
RECON_TYPE	optical section series/mosaic
THUMBNAIL	P1187/asyn6_vt.jpg
VOLUME_DIMENSION	11286, 15282, 1
VOLUME_NAME	asyn6/asyn6_montage.tif
VOXEL_SCALE	.36, .36, 2.5
RECONSTRUCTION_IMAGES_ID	30
NEUROINFORMATICA_URL	http://ccdb-aims.ucsd.edu:8880/showMe.jsp?instGUID=A6A61340-20F0-DF73-BA25-9E5B0E26BB8E
RECON_IMAGE_DESC	Image mosaic of a section through striatum showing localization of alpha synuclein in a wild type mouse. Dark area in upper right (cortex) results from uneveness in section flatness rather than lack of staining in this area
RECON_FILE_NAME	asyn6/asyn6_wt.jpg
VOLUME_THUMBNAIL	P1187/asyn6_vt.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