## **Cell Centered Database**

# University of California, San Diego Maryann Martone

### Microscopy Product #:3427 Phaeo22

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Image2D	Reconstruction	Segmentation

## **Project Information:**

PROJECT ID	P1576
PROJECT_NAME	Chloroplast Ultrastructure of Phaeocystis antarctica in High and Low Light Conditions
PROJECT_DESCRIPTION	The three-dimensional morphological rearrangements for two conditions that mimic light conditions for the Antarctic summer and winter were studied in Phaeocystis antarctica Karsten
LEADER	Tiffany Moisan
FUNDING_AGENCY	National Aeronautics and Space Administration
PROJECT_START_DATE	
PROJECT_END_DATE	
COLLABORATORS	Gina Sosinsky, Casey Buitenhuys, Mark Ellisman
PUBLICATION1	Moisan, T., Ellisman, M. H., Buitenhuys, C.W., Sosinsky, G. E., (2006) Differences in Chloroplast Ultrastructure of Phaeocystis antarctica in High and Low Light Conditions, Marine Blology, 149 (6) 1281-1290
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	To examine thylakoid membrane architecture under conditions of high light
	High light condition
EXPERIMENTER	Tiffany Moisan
EXPERIMENT_NAME	
EXPERIMENT_DATE	

Subject Information -	
GROUP_BY	Light level
SUBJECT_NAME	High light
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	Phaeocystis antarctica
SPECIES	algae
STRAIN	Karsten
AGE	days
AGECLASS	6-8 generation cultured cells
ANIMAL_NAME	
LITTER_ID	
SEX	unspecified
VENDOR	
WEIGHT	grams

Tissue -	
ANATOMIC_LOCATION	
MICROTOME	Ultramicrotome
ORIENTATION	
THICKNESS	.25 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	P1576_Phaeo1.xml
TISSUE_GROUP_TYPE	

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	3427
IMAGE_BASENAME	Phaeo22
CREATE_DATE	
INSTRUMENT	JEOL 4000EX IVEM
MICROSCOPE_TYPE	IVEM
PLANE_COUNT	
PRODUCT_TYPE	SINGLE TILT
PURL	
SESSION_NAME	
TELESCIENCE_SRB	P1576/Experiment_3363/Subject_61/Tissue_75/Microscopy_3427
X_RESOLUTION	nm/pixels
Y_RESOLUTION	nm/pixels
XSIZE	
YSIZE	

### **Protocol:**

Culture conditions. Cultures of colonial P. antarctica (CCMP 1374) were grown semi-continuously for 5-8 generations in f/2 medium (Guillard and Ryther 1962) under continuous blue light at 4¿C at irradiances of 14 and 259 ¿mol quanta m-2 s-1.

Specific growth rate. Specific growth rate was estimated by a linear regression of loge transformed daily determinations of in vivo

fluorescence intensity (n=2) measured with a Turner Model 10 fluorometer.

Sample preparation for electron microscopy. P. antarctica colonies were fixed on ice with a 2% glutaraldehyde and 1.3% osmium tetroxide solution for 30 minutes and rinsed in distilled water. Cells were dehydrated through a series of ethanol: water washes (25:75, 50:50, 75:25, 95:5), three 100% ethanol washes and finally through three washes of 100% acetone. Cells were pelleted and fixed in an Epon resin. The fixation process lends itself to a breakup of the colonial matrix and we were able to examine P. antarctica individual colonial cells using electron tomography. Embedded samples were cut on a Reichert-Jung Ultracut E microtome, transferred to 50/50 mesh copper clam grids, and stained with uranyl acetate and lead citrate. After staining, 20 nm colloidal gold particles (Sigma-Aldrich Chemicals, St. Louis, MO) were added to both sides of the grid to serve as fiducial markers for aligning tilted images. Individual colonial cells were observed at low magnification at 80kV on a JEOL 100CX to determine specimen quality and to select suitable samples.

Intermediate voltage electron microscopy. Sections of 0.25 (high light condition) and 0.75 ¿m (low light condition) in thickness were cut, post-stained with uranyl acetate and lead citrate and examined at 400 kV on a JEOL 4000 intermediate voltage electron microscope. Tilt series consisting of 61 images (-60¿ to 60¿ at 2¿ tilt increments) were collected at either 12-15,000 magnification (low light condition) or 20-30,000 magnification (high light condition). Images were collected on film (Kodak 4489 electron image film) or on a Slow-Scan Cooled CCD camera (Fan et al. 2000). Sections were pre-irradiated before each tilt series in order to limit anisotropic specimen thinning during specimen examination (Luther 1992). The illumination was held constant using parallel electron beam conditions and the image was maximized for each exposure. A computer-controlled goniometer was used to accurately tilt the specimen. For tilt series acquired on film, digitization was accomplished using a Photometrics 1024 x 1024 Cooled CCD camera containing a 19-¿m2 pixel with sampling sizes of ~50-85 ¿m pixel-1.

Single-axis tilt series tomographic reconstruction methodology. Tilted images were aligned with each other by use of a set of common fiducial marks consisting of 20 nm colloidal gold beads. Reconstruction methods follow that those of Perkins et al. (1997). The common fiducial marks on each image of the tilt series were aligned using the program XFIDO. Alignment of the tilt series was initially calculated using a least-squares algorithm through the z-direction of the tilt series using the program SAXALIGN. After initial alignment, volumes were computed using either a standard r-weighted simple back projection algorithm or a Globus enabled parallelized version of this algorithm that considerably speeded up these computations (Smallen et al. 2000).

The 3D reconstruction is viewed and analyzed with ANALYZE AVW (Biomedical Imaging Resource, Mayo Clinic, http://www.mayo.edu/bir/Software/Analyze/Analyze.html). Individual thylakoids, pyrenoids, and chloroplast membranes were traced on the electron tomographic reconstruction using the program XVOXTRACE. The resolution of the organelles was estimated to be ~10 nm (based on detectability of features and pixel sampling criteria). All computations and graphics were performed on either Silicon Graphics or Sun workstations.

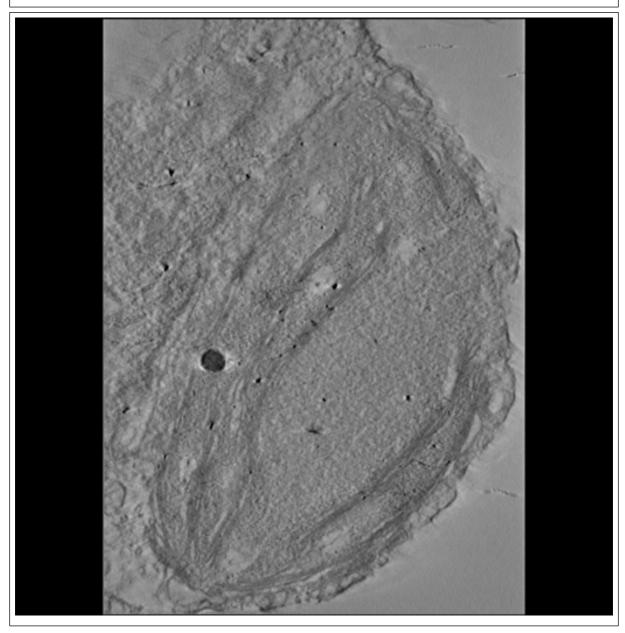
Image Type -	
SINGLE_TILT_IMAGE_SEQ_ID	6005
TILT_INCREMENT	2 degrees
SINGLE_TILT_NOTES	Specimen was preirradiated prior to imaging
SINGLETILTIMAGESEQ_ID	6005
TILT_INCREMENT	2 degrees
RANGE_MAX	60 degrees
RANGE_MIN	-60 degrees
SINGLE_NOTES	Specimen was preirradiated prior to imaging

Specimen Description -	
ANATOMICAL_DETAIL	6011
ATLAS_COORD	, ,
CELL_TYPE	algae
STRUCTURE	chloroplast

Electron Microscopy Product -	
EM_PRODUCT_ID	6008
ACCELERATING_VOLTAGE	400 KeV
EMBEDDING_MEDIUM	resin
MAGNIFICATION	30000
RECORDING_MEDIUM	film

## Reconstruction

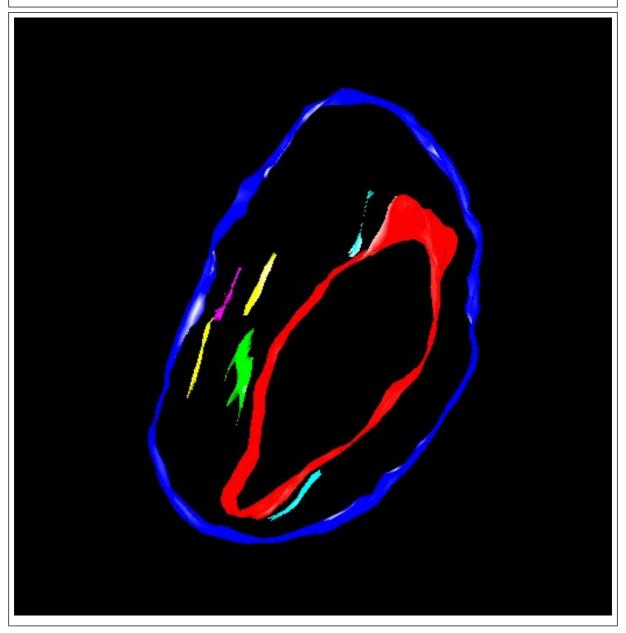
### Reconstruction Image -



Reconstruction -		
RECONSTRUCTION3D_ID	6009	
ALIGNMENT_METHOD	manual	
ALIGNMENT_PROGRAM	xfido, saxalign	
CROPPING_COORDINATE1	,	
CROPPING_COORDINATE2	,	
RECON_ALGORITHM	R-weighted back projection	
RECON_DESC	Zip file containing the tomographic volume in Analyze 7.5 format (Phaeo22_oblique68.img/hdr). Volume was resectioned along an oblique axis to "flatten" the section prior to tracing using the oblique section function in Analyze. The file containing the manual segmentation is also included (.trace).	
RECON_PROGRAM	Suprim	
RECON_TYPE	single tilt electron tomography	
VOLUME_DIMENSION	624, 884, 68	
VOLUME_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/phaeo22_vol.zip	
VOXEL_SCALE	, ,	
RECONSTRUCTION_IMAGES_I	6009	
RECON_IMAGE_DESC	Single computed slice through a tomographic volume of the chloroplast of a blue green algae grown under high light conditions.	
RECON_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/phaeo22_vol.jpg	
VOLUME_THUMBNAIL	P1576/phaeo22_vol_thmb.jpg	
ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/phaeo22_vol.mov	
ANIMATION_FILE_FORMAT	Quicktime	
ANIMATION_DESC	Animation through the computed slices of a tomographic volume of the chloroplast of a blue green algae grown under high light conditions.	

# Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	6017
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format (*.synu) and the Viewdata file required for viewing.  Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and some of the thylakoid membranes were segmented.
IS_MANUAL	Υ
LABELING_RANK	none
NUMBER_OF_OBJECT	0
OBJECT_DESC	thylakoid membranes traced as a continous object
OBJECT_NAME	green
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg
SEGMENTED_OBJECT_ID	6017
SEGMENT_PERSON_NAME	Raina Taitano
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg
SEGMENTED_OBJECT_ID	6015
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format (*.synu) and the Viewdata file required for viewing.
	Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and some of the thylakoid membranes were segmented.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
OBJECT_DESC	thylakoid membrane
OBJECT_NAME	cyan
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg

Segmentation -	
SEGMENTED_OBJECT_ID	6015
SEGMENT PERSON NAME	Raina Taitano
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg
SEGMENTED_OBJECT_ID	6016
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format (*.synu) and the Viewdata file required for viewing.  Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and some of the thylakoid membranes were segmented.
IS MANUAL	Υ
LABELING_RANK	none
NUMBER OF OBJECT	0
OBJECT DESC	thylakoid membrane
OBJECT_NAME	cyanne
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg
SEGMENTED_OBJECT_ID	6016
SEGMENT PERSON NAME	Raina Taitano
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg
SEGMENTED_OBJECT_ID	6018
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format (*.synu) and the Viewdata file required for viewing.  Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and
	some of the thylakoid membranes were segmented.
IS_MANUAL	Υ
LABELING_RANK	none

Segmentation -		
NUMBER_OF_OBJECT	0	
OBJECT_DESC	thylakoid membrane	
OBJECT_NAME	mag	
OBJECT_TYPE	surface	
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg	
SEGMENTED_OBJECT_ID	6018	
SEGMENT_PERSON_NAME	Raina Taitano	
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu	
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip	
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg	
SEGMENTED_OBJECT_ID	6019	
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.	
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format (*.synu) and the Viewdata file required for viewing.  Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and	
IO MANULAL	some of the thylakoid membranes were segmented.	
IS_MANUAL	Y	
LABELING_RANK NUMBER_OF_OBJECT	none	
	0 pyrapaid	
OBJECT_DESC	pyrenoid	
OBJECT_NAME	nucleus	
OBJECT_TYPE	surface /tologgiongo/homo/CCDR_DATA_USER_portal/P1576/Experiment_3	
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg	
SEGMENTED_OBJECT_ID	6019	
SEGMENT_PERSON_NAME	Raina Taitano	
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu	
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip	
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg	
SEGMENTED_OBJECT_ID	6020	
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.	
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format	

Segmentation -	
<u>oogmonation</u>	(*.synu) and the Viewdata file required for viewing.
	Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and some of the thylakoid membranes were segmented.
IS_MANUAL	Υ
LABELING_RANK	none
NUMBER_OF_OBJECT	0
OBJECT_DESC	chloroplast membrane
OBJECT_NAME	outterwall
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg
SEGMENTED_OBJECT_ID	6020
SEGMENT_PERSON_NAME	Raina Taitano
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg
SEGMENTED_OBJECT_ID	6021
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format (*.synu) and the Viewdata file required for viewing.
	Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and some of the thylakoid membranes were segmented.
IS_MANUAL	Υ
LABELING_RANK	none
NUMBER_OF_OBJECT	0
OBJECT_DESC	thylakoid membrane
OBJECT_NAME	yell
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg
SEGMENTED_OBJECT_ID	6021
SEGMENT_PERSON_NAME	Raina Taitano
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg

Segmentation -		
SEGMENTED_OBJECT_ID	6022	
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast outer membrane (blue), the pyrenoid (red) and some of the thylakoid membranes (multiple colors) from a tomographic reconstruction of a blue green algae grown in high light conditions.	
DOWNLOADABLE_FILE_DESC	Zip file containing the trace file with the manual traces (Phaeo22.trace) along with the surfaced objects in Synu format (*.synu) and the Viewdata file required for viewing.  Curator's note: The segmented objects were not well documented but it appears that the outer chloroplast membrane, the pyrenoid and	
	some of the thylakoid membranes were segmented.	
IS_MANUAL	Y	
LABELING_RANK	none	
NUMBER_OF_OBJECT	0	
OBJECT_DESC	thylakoid membrane	
OBJECT_NAME	yellow	
OBJECT_TYPE	surface	
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.jpg	
SEGMENTED_OBJECT_ID	6022	
SEGMENT_PERSON_NAME	Raina Taitano	
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes using Xvoxtrace 2.8 followed by surfacing with Synu	
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3 363/Subject_61/Tissue_75/Microscopy_3427/Phaeo22_seg.zip	
THUMBNAIL	P1576/phaeo22_seg_thmb.jpg	

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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