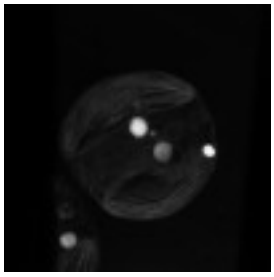
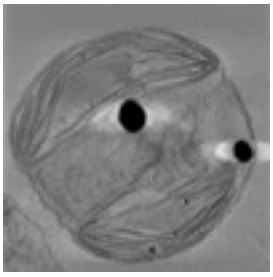
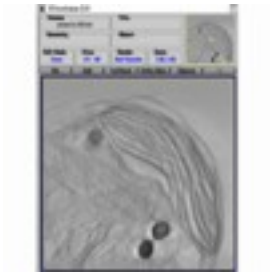
Cell Centered Database

University of California, San Diego

Maryann Martone

Microscopy Product #:3410 Phaeo5

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=3410

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 BIology, 149 (6) 1281-1290
PUBLICATION2	
PUBLICATION3	

Experiment Information -	
PURPOSE	To examine the architecture of thylakoid membranes in algae grown under low light conditions
TITLE	Low light condition
EXPERIMENTER	Tiffany Moisan
EXPERIMENT_NAME	
EXPERIMENT_DATE	

Subject Information -	
GROUP_BY	Light level
SUBJECT_NAME	Low light
FIXATION_METHOD_ID	
SCIENTIFIC_NAME	Phaeocystis antarctica
SPECIES	Algae
STRAIN	Karsten
AGE	days
AGECLASS	8 generations
ANIMAL_NAME	
LITTER_ID	
SEX	unspecified
VENDOR	
WEIGHT	grams

Tissue -	
ANATOMIC_LOCATION	
MICROTOME	Ultramicrotome
ORIENTATION	
THICKNESS	.75 um
TISSUE_PROD_STORAGE	
EXTERNAL_FILE_NAME	P1576_Phaeo1.xml
TISSUE_GROUP_TYPE	

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	3410
IMAGE_BASENAME	Phaeo5
CREATE_DATE	
INSTRUMENT	JEOL 4000EX IVEM
MICROSCOPE_TYPE	IVEM
PLANE_COUNT	
PRODUCT_TYPE	SINGLE TILT
PURL	
SESSION_NAME	
TELESCIENCE_SRB	P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410
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	2002
TILT_INCREMENT	2 degrees
SINGLE_TILT_NOTES	The specimen was pre-irradiated prior to taking the tilt series
SINGLETILTIMAGESEQ_ID	2002
TILT_INCREMENT	2 degrees
RANGE_MAX	60 degrees
RANGE_MIN	-60 degrees
SINGLE_NOTES	The specimen was pre-irradiated prior to taking the tilt series

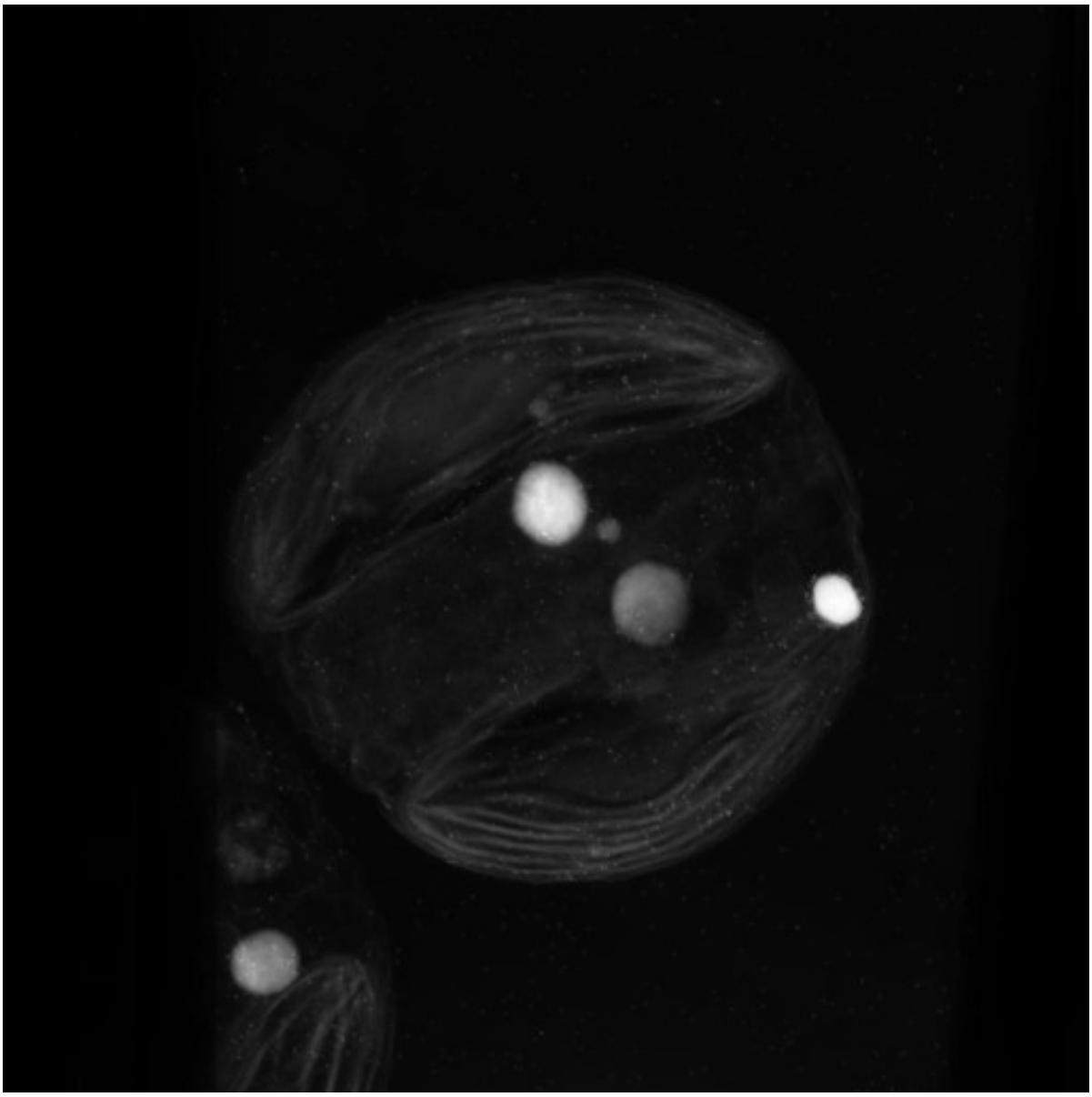
Specimen Description -	
ANATOMICAL_DETAIL	2003
ATLAS_COORD	, ,
CELL_TYPE	algae

Specimen Description -	
STRUCTURE	chloroplast

Electron Microscopy Product -	
EM_PRODUCT_ID	2004
ACCELERATING_VOLTAGE	400 KeV
MAGNIFICATION	20000
RECORDING_MEDIUM	film
EM_NOTES	Check the magnification on this, because the original negatives are not available.

Raw 2D Image

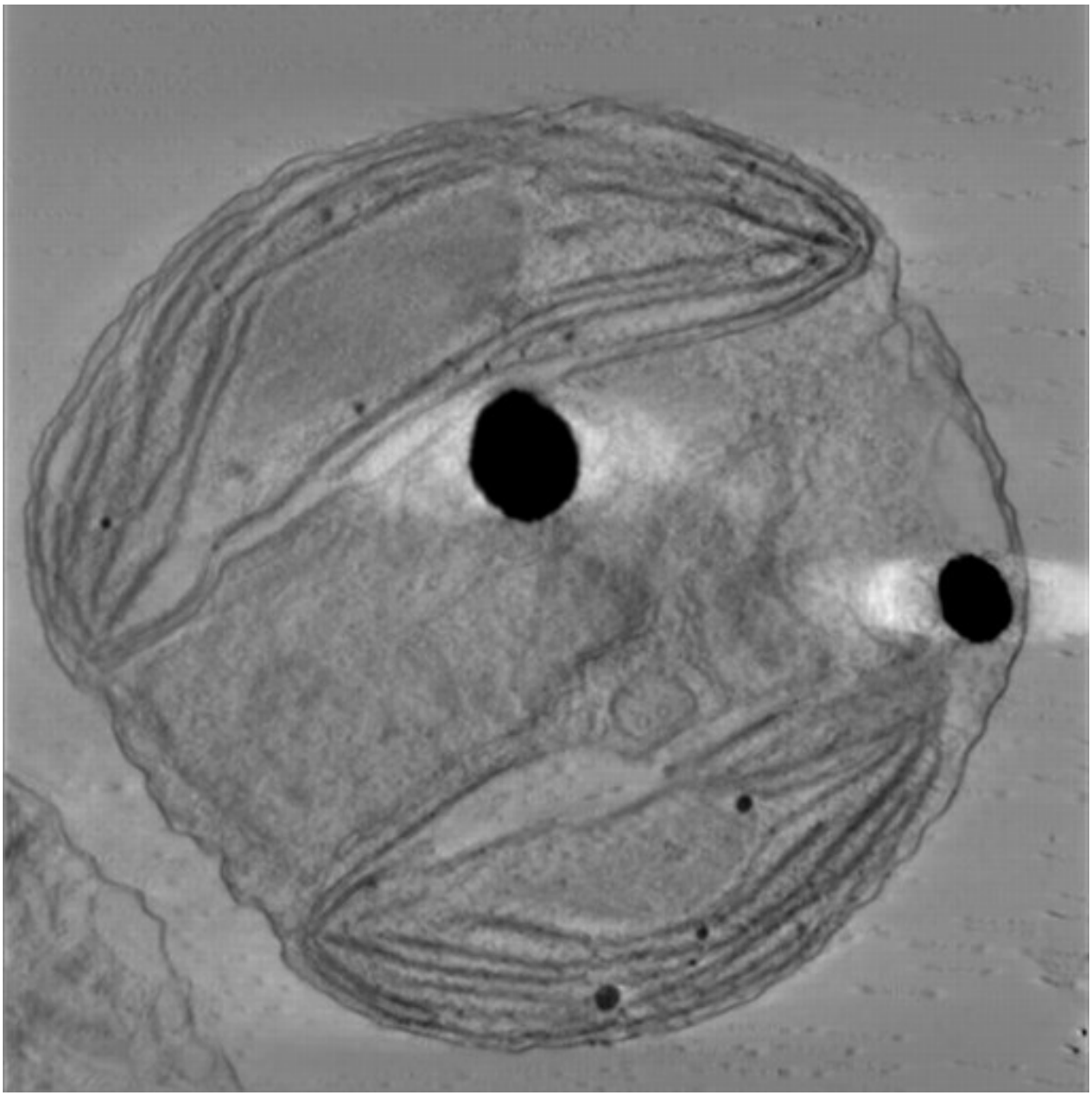
Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	3004
BIT_DEPTH	14 bit
DIGITIZED_BY	Tiffany Moisan
DIGITIZING_PLATFORM	Photometrics 1024x1024 cooled CCD camera with 19 sq um pixels.
IMAGE_DESC	Tar file containing compressed tilt images in suprim format (*.f.Z), the aligned tilt image (*.s.Z) along with the fiducial mark files produced by xFido (phaeo5.fido)
IMAGE_FILE_FORMAT	Suprim
IMAGE_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_img.jpg
RAW_ANIMATION_DESC	Animation of aligned tilt series of a blue green algae grown in low light conditions imaged with intermediate voltage electron microscopy, showing the chloroplasts and thylakoid membranes. Contrast is reversed so that electron dense structures appear bright.
RAW_ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_good_align.mov
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_img.tar
THUMBNAIL_DESC	Single tilt image from a tomographic series of a blue green algae grown in low light conditions imaged with intermediate voltage electron microscopy, showing the chloroplasts and thylakoid membranes. Contrast is reversed so that electron dense structures appear bright.
THUMBNAIL_FILE	P1576/phaeo5_img_thmb.jpg
X_SIZE	1024 pixels
Y_SIZE	1024 pixels
NOTES	Check the resolution because there are a range reported in the manuscript

Reconstruction

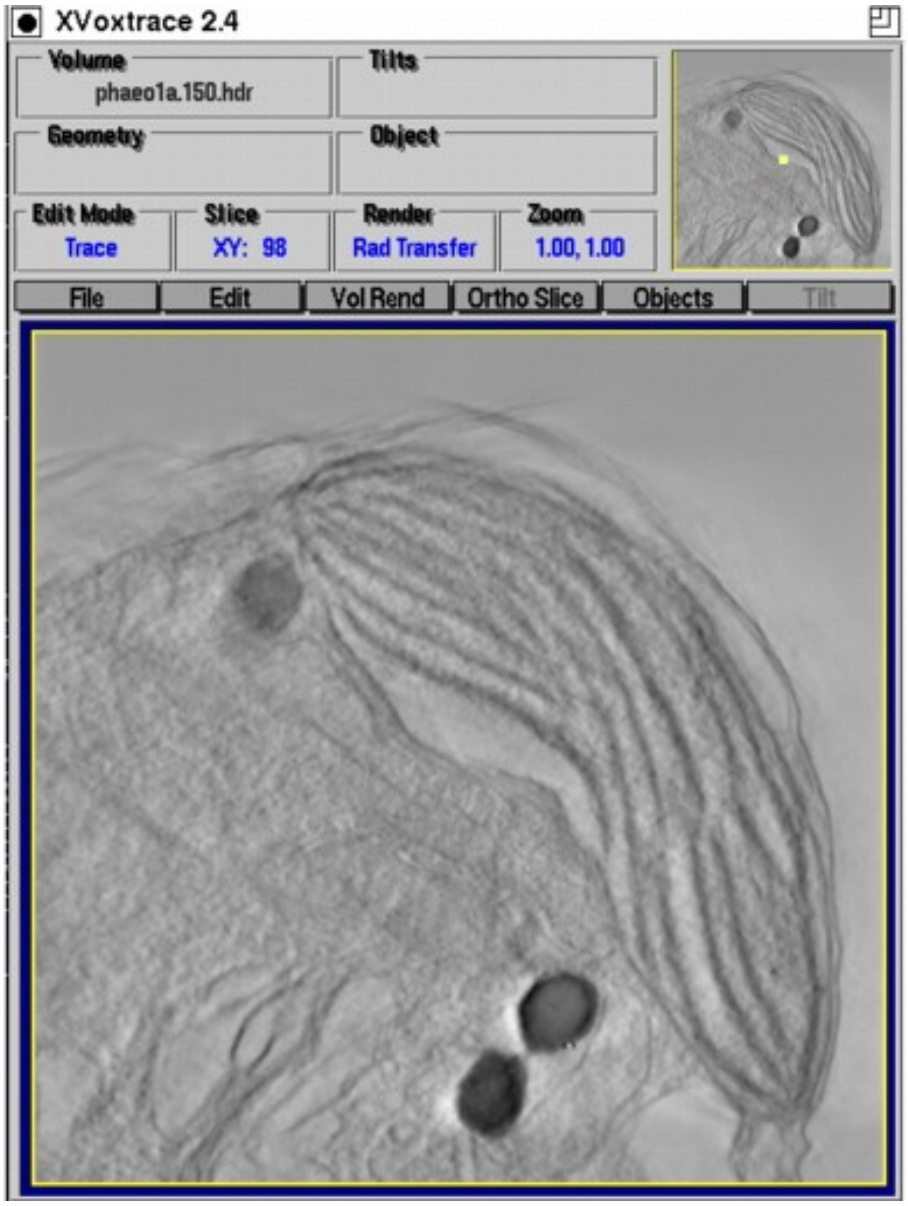
Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	2004
ALIGNMENT_METHOD	manual
ALIGNMENT_PROGRAM	xfido, saxalign
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
RECON_ALGORITHM	R-weighted back projection
RECON_DESC	Tar file containing compressed volume files in Analyze 7.5 format. Both the .img and .hdr file are included. Multiple versions of the volume are present, each with a different number of z slices, as indicated by the number in the file name.
RECON_PROGRAM	Suprim
RECON_TYPE	single tilt electron tomography
VOLUME_DIMENSION	652, 652, 200
VOLUME_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_vol.jpg
VOXEL_SCALE	, ,
RECONSTRUCTION_IMAGES_ID	2004
RECON_IMAGE_DESC	Single computed slice through tomographic volume of blue green algae exposed to low light conditions. Reconstruction shows the chloroplast and inner thylakoid membrane. Some electron dense structures that look like lipid bodies are also visible.
RECON_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_vol.jpg
VOLUME_THUMBNAIL	P1576/phaeo5_vol_thmb.jpg
ANIMATION_FILE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_good_vol.mov
ANIMATION_FILE_FORMAT	Quicktime
ANIMATION_DESC	Animation through computed slices of tomographic reconstruction of a blue green algae cell grown in low light conditions

Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	2007
DISPLAY_IMAGE_DESC	Screenshot of manual segmentation using Xvoxtrace of the thylakoid and chloroplast membranes
DOWNLOADABLE_FILE_DESC	Tar file containing Xvoxtrace file with manual contours.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	1
OBJECT_DESC	chloroplast outer membrane
OBJECT_NAME	chloroplastmembrane
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_seg.jpg
SEGMENTED_OBJECT_ID	2007
SEGMENT_PERSON_NAME	Casey Buitenhuys
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes, including contact sites, using Xvoxtrace 2.9
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3409/phaeo4_seg.tar
THUMBNAIL	P1576/phaeo5_seg_thmb.jpg
SEGMENTED_OBJECT_ID	2008
DISPLAY_IMAGE_DESC	Screenshot of manual segmentation using Xvoxtrace of the thylakoid and chloroplast membranes
DOWNLOADABLE_FILE_DESC	Tar file containing Xvoxtrace file with manual contours.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	1
OBJECT_NAME	pyrenoid
OBJECT_TYPE	contour
SEGMENTED_OBJECT_ID	2008
SEGMENT_PERSON_NAME	Casey Buitenhuys
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes, including contact sites, using Xvoxtrace 2.9
THUMBNAIL	P1576/phaeo5_seg_thmb.jpg
SEGMENTED_OBJECT_ID	2009
DISPLAY_IMAGE_DESC	Screenshot of manual segmentation using Xvoxtrace of the thylakoid and chloroplast membranes
DOWNLOADABLE_FILE_DESC	Tar file containing Xvoxtrace file with manual contours.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	1
OBJECT_DESC	thylakoid membranes traced as a continous object
OBJECT_NAME	thylakoid
OBJECT_TYPE	contour
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3

Segmentation -	
	362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_seg.jpg
SEGMENTED_OBJECT_ID	2009
SEGMENT_PERSON_NAME	Casey Buitenhuys
SEG_DESC	Manual segmentation of the chloroplast membrane and thylakoid membranes, including contact sites, using Xvoxtrace 2.9
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3410/phaeo5_seg.tar
THUMBNAIL	P1576/phaeo5_seg_thmb.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