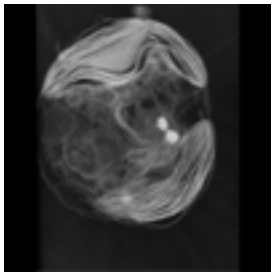
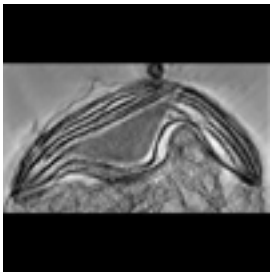
Cell Centered Database

University of California, San Diego

Maryann Martone

Microscopy Product #:3406 Phaeo1

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=3406

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	3406
IMAGE_BASENAME	Phaeo1
CREATE_DATE	
INSTRUMENT	JEOL 4000EX IVEM
MICROSCOPE_TYPE	IVEM
PLANE_COUNT	61
PRODUCT_TYPE	SINGLE TILT
PURL	
SESSION_NAME	
TELESCIENCE_SRB	P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3406
X_RESOLUTION	nm/pixels
Y_RESOLUTION	nm/pixels
XSIZE	1024
YSIZE	1024

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	5020
TILT_INCREMENT	2 degrees
SINGLET_DESC	Specimen was pre-irradiated before imaging
SINGLE_TILT_NOTES	Specimen was pre-irradiated before imaging
SINGLETILTIMAGESEQ_ID	5020
TILT_INCREMENT	2 degrees
RANGE_MAX	60 degrees
RANGE_MIN	-60 degrees
SINGLET_DESC	Specimen was pre-irradiated before imaging
SINGLE_NOTES	Specimen was pre-irradiated before imaging

Specimen Description -	
ANATOMICAL_DETAIL	5100

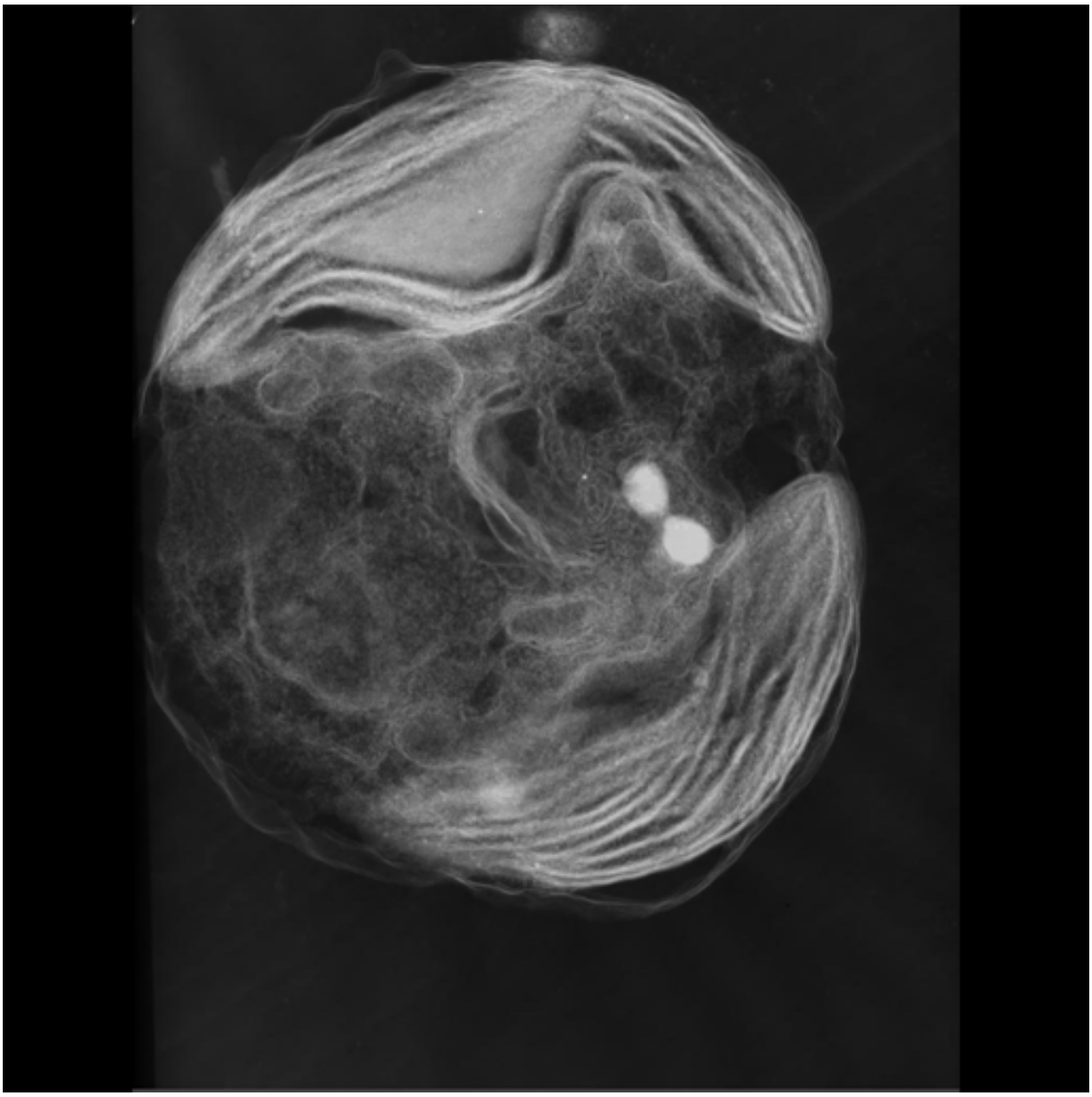
Specimen Description -

ATLAS_COORD	, ,
CELL_TYPE	algae
STRUCTURE	chloroplast

Electron Microscopy Product -	
EM_PRODUCT_ID	5100
ACCELERATING_VOLTAGE	400 KeV
EMBEDDING_MEDIUM	resin
MAGNIFICATION	12000
RECORDING_MEDIUM	film
EM_NOTES	Some of the datasets were taken at 12000 and some at 15000. We will need to check this against the negatives.

Raw 2D Image

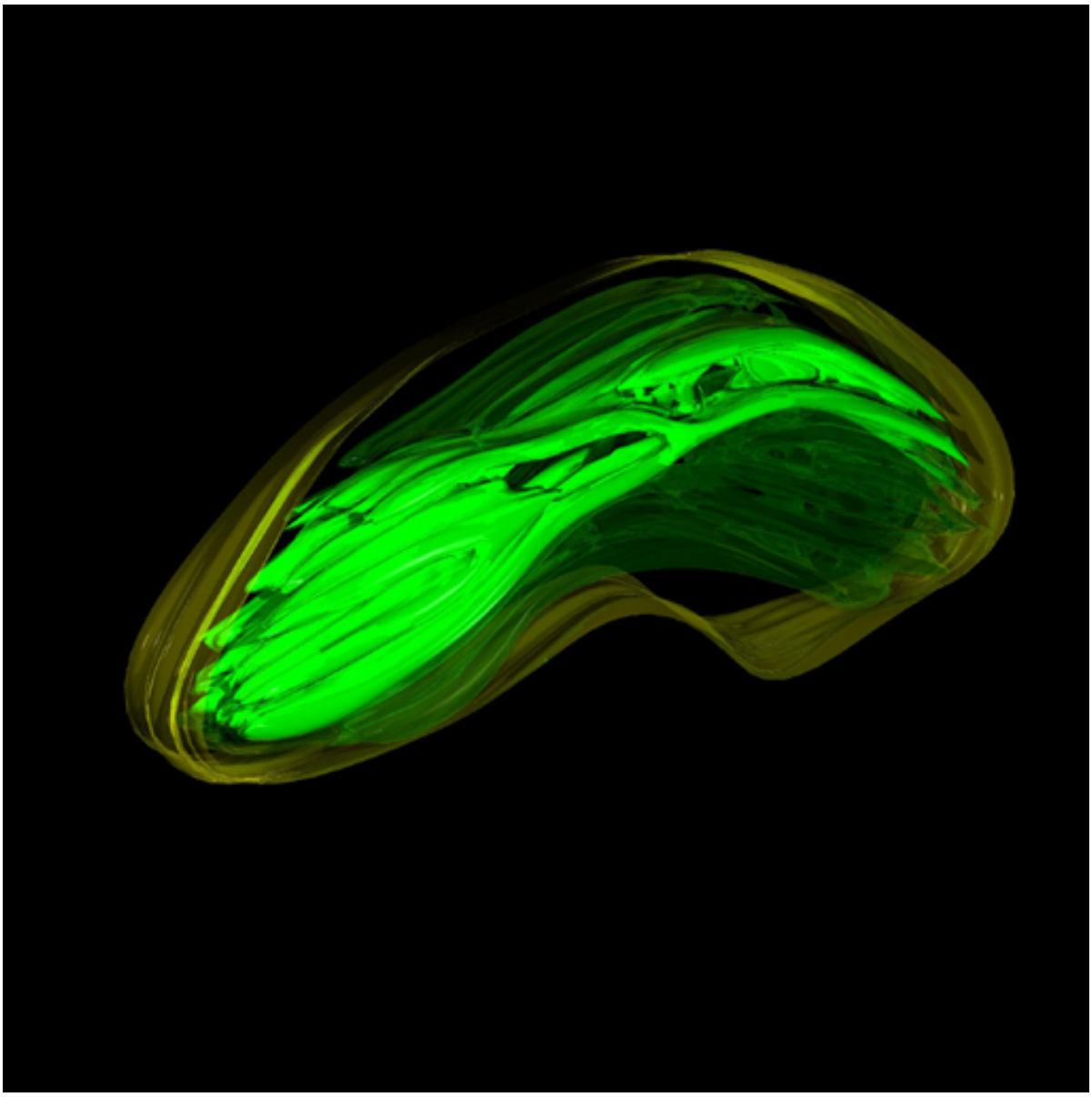
Raw Low Resolution 2D Image -



Raw 2D Image -	
IMAGE2D_ID	3009
BIT_DEPTH	14 bit
DIGITIZING_PLATFORM	Photometrics 1024x1024 cooled CCD camera with 19 sq um pixels.
IMAGE_DESC	Tar file containing digitized full unaligned resolution images (*.f), along with the fiducial mark file (*.fido), the origin file (*.fido,origin) and the file containing the angles used in the reconstruction.
IMAGE_FILE_FORMAT	Suprim
IMAGE_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3406/phaeo1_img.jpg
RAW_DATA_FILE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3406/phaeo1_img.tar
THUMBNAIL_DESC	Single tilt image at zero degrees tilt of a 0.5 thick section of blue green algae grown under low light conditions showing the chloroplast and thylakoid membranes taken with intermediate voltage electron microscopy. Contrast is reversed so that electron dense structures appear bright.
THUMBNAIL_FILE	P1576/phaeo1_img_thmb.jpg
X_SIZE	1024 pixels
Y_SIZE	1024 pixels
NOTES	Check magnification

Segmentation

Segmentation Image -



Segmentation -	
SEGMENTED_OBJECT_ID	5121
ANALYZE_DESC	The contours of the chloroplast and thylakoid membranes were manually traced using Xvoxtrace 2.1 and then the contours were surfaced using both the marching cube (contain mcube in the file name) and nuages algorithms through Synu. Synu output was converted into Amira format (.iv) using routines developed at NCMIR. Volumes were generated for each of the segmented structures using Synu in Analyze 7.5 format.
ANALYZE_DESC	The contours of the chloroplast and thylakoid membranes were manually traced using Xvoxtrace 2.1 and then the contours were surfaced using both the marching cube (contain mcube in the file name) and nuages algorithms through Synu. Synu output was converted into Amira format (.iv) using routines developed at NCMIR. Volumes were generated for each of the segmented structures using Synu in Analyze 7.5 format.
DISPLAY_IMAGE_DESC	Surface rendering of the chloroplast membrane (yellow) and thylakoid membranes (green) from a blue green algae grown under low light conditions. In some cases, thylakoid membranes formed branching tree-like structures (highlighted in light green).
DOWNLOADABLE_FILE_DESC	Tar file containing segmented chloroplast membrane and thylakoids in Synu (.synu), Amira (.iv) and Analyze 7.5 (.img/.hdr) format. File also contains the Xvoxtrace .trace file.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
OBJECT_DESC	thylakoid membranes
OBJECT_NAME	thylakoid
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3406/phaeo1_seg.jpg
SEGMENTED_OBJECT_ID	5121
SEGMENT_PERSON_NAME	Casey Buitenhuys
SEG_DESC	Segmentation of chloroplast outer membrane (yellow) and thylakoid inner membranes (green) followed by surfacing using Amira
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3406/phaeo1_seg.tar
SEGMENTED_OBJECT_ID	5120
ANALYZE_DESC	The contours of the chloroplast and thylakoid membranes were manually traced using Xvoxtrace 2.1 and then the contours were surfaced using both the marching cube (contain mcube in the file name) and nuages algorithms through Synu. Synu output was converted into Amira format (.iv) using routines developed at NCMIR. Volumes were generated for each of the segmented structures using Synu in Analyze 7.5 format.
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Segmentation -	
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DOWNLOADABLE_FILE_DESC	Tar file containing segmented chloroplast membrane and thylakoids in Synu (.synu), Amira (.iv) and Analyze 7.5 (.img/.hdr) format. File also contains the Xvoxtrace .trace file.
IS_MANUAL	Y
LABELING_RANK	none
NUMBER_OF_OBJECT	0
OBJECT_DESC	chloroplast membranes
OBJECT_NAME	chloroplast
OBJECT_TYPE	surface
SEGMENTED_OBJ_2D_IMAGE	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3406/phaeo1_seg.jpg
SEGMENTED_OBJECT_ID	5120
SEGMENT_PERSON_NAME	Casey Buitenhuys
SEG_DESC	Segmentation of chloroplast outer membrane (yellow) and thylakoid inner membranes (green) followed by surfacing using Amira
SEG_FILE_NAME	/telescience/home/CCDB_DATA_USER.portal/P1576/Experiment_3362/Subject_60/Tissue_74/Microscopy_3406/phaeo1_seg.tar

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