

## Fine structure of Cyanobacteria, *Spirulina platensis* and *Spirulina subsalsa*, as viewed by x-ray microscope, XM-1, beamline 6.1.2.

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*Spirulina platensis* (*Arthrospira*) [1] and *Spirulina subsalsa* [1] were examined for their fine structure in the initial stages of a project to obtain high-resolution images of these spiral cyanobacteria and the spiral chloroplasts of *Spirogyra species*. These high-resolution images will allow a close comparison between morphological features of prokaryotic cells and the eukaryotic photosynthetic organelle. We are looking for high resolution of features along the edges of the organisms and interior to the cells. Live specimens were used to avoid artifacts introduced by chemical preservation. Specimens are quickly frozen in their nutrient media to obtain the freshest possible images. Cryo-techniques are being developed with the cryo-stage of XM-1, the x-ray microscope at beamline 6.1.2 of the Advanced Light Source. The XM-1 was built and is operated by the Center for X-ray Optics. The cryo-stage provides high resolution with long exposures and mitigates the side effects of radiation damage. We froze live specimens at quick freeze rates to provide the protective matrix of frozen water in an amorphous state and avoid ice crystallization. Freeze rates were at least  $-5000$  degrees Celsius per second. The final temperature goal is about  $-130$  °C. At this temperature, the time scale for crystallization is so long that no development is noticeable during the experiment session.

Several cyanobacterial cultures were brought from Mills College [2, 3]. Figs. 1 and 4 image the bright, blue-green cylindrical filaments of *Spirulina platensis* (*Arthrospira*). It had a lazy spiral turn, filament width 5-6  $\mu\text{m}$ . Cell end walls are clearly visible crossing the filament at intervals of about 2-3  $\mu\text{m}$ . No fimbriae (fimbrillins are hydrophobic amino acids arranged in 5-7 nm diameter tubules) were observed extending perpendicularly from the bacterial cell surface [expected from ref 1]. Fig. 1 is most like *Spirulina platensis* (*Arthrospira*) of Rippka [1] although labeled in image files as *S. subsalsa*. The image reveals two interesting internal structures: spherical shapes and looping strands with the appearance of beads-on-a-string. Both are visible in the 2<sup>nd</sup> cell from the top of the micrograph. The cells in the lower third of the micrograph appear to have recently completed cell division. We reach this conclusion because the cross wall is very thin and the two cells are shorter (1  $\mu\text{m}$ ) than neighbor cells. Structures, which also have the appearance of beads-on-a-string, are clustered within these two cells at their common wall.

*Spirulina subsalsa*, Fig. 2, has a very tightly coiled spiral filament about 1-2  $\mu\text{m}$  in diameter. As expected from Rippka's description of visible-light images [1] no cross walls are apparent dividing cells along the length of the spiral. A series of linear striations, however, does appear parallel to the lengthwise, spiral axis. No fine extensions of cellular matrix are apparent in the surrounding medium where we might have expected to see fimbriae [1, 4].

We plan to view the much larger cells of *Spirogyra*, which are 20-40  $\mu\text{m}$  in cylindrical diameter. They grow into very long filaments of individual cells, each contain a spiral chloroplast (or two at times of replication).

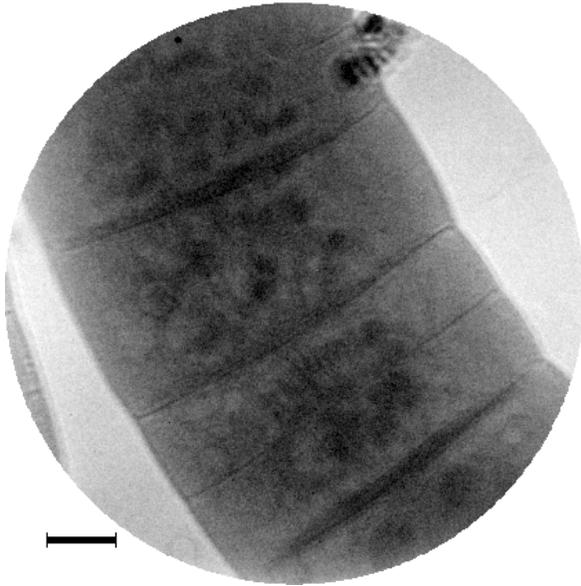


Fig. 1 *Spirulina platensis* XM-1 image from beamline 6.1.2. Frozen ( $-80\text{ }^{\circ}\text{C}$ ) in seawater nutrient solution. Sandwiched between two silicon nitride windows (100 nm thick), 4  $\mu\text{l}$  of sample solution, 10  $\mu\text{m}$  total thickness. End walls between contiguous cylindrical cells are clearly visible, as expected for *Spirulina platensis* (*Arthrospira*) of Rippka [1]. Some internal structures are apparent within the cells; beads-on-a-string shapes are visible in cells 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> from the top of the image. Magnification is 2400x. Good freeze, free of crystals. Scale bar is 1  $\mu\text{m}$ .

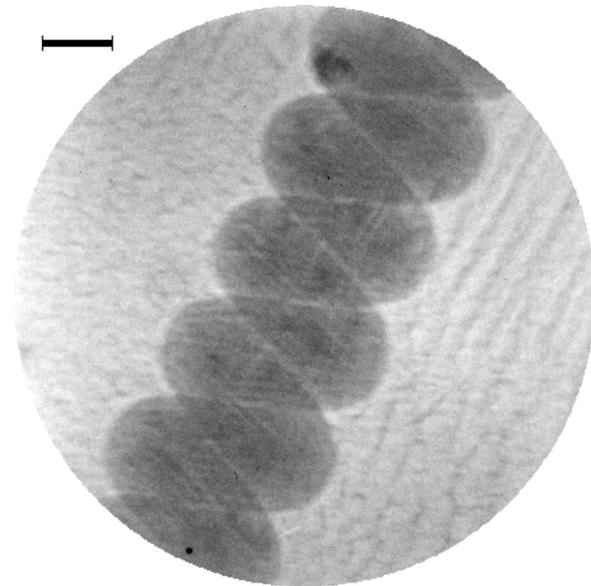


Fig. 2 *Spirulina subsalsa*, (This culture is originally from Carolina Biological supplies [3]), cyanobacteria culture Mills College [2]. Tight coils visible. Looks like *S. subsalsa* [3], which is similar to PCC 6313 of Rippka in lacking visible end walls [1]. No end walls appear to cross the width of the spiral. Length-wise striations appear. The specimen is in seawater from a live preparation of *Spirulina subsalsa*. Quickly frozen to  $-140\text{ }^{\circ}\text{C}$  then stabilized at  $-80\text{ }^{\circ}\text{C}$ , 2.4 nm wavelength, 2400x magnification. Specimen is sandwiched between two silicon nitride windows (100 nm thick), 4  $\mu\text{l}$  of sample solution, 10  $\mu\text{m}$  total thickness. Scale bar 1  $\mu\text{m}$ .

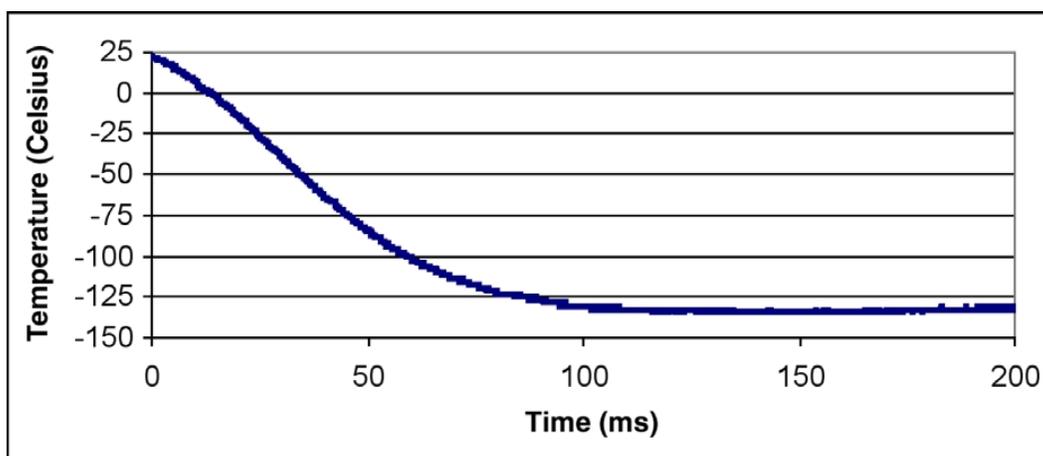


Fig. 3 Temperature drop at sample during freezing process. The sample goes from 0 Celsius to  $-100$  Celsius in about 35 milliseconds.

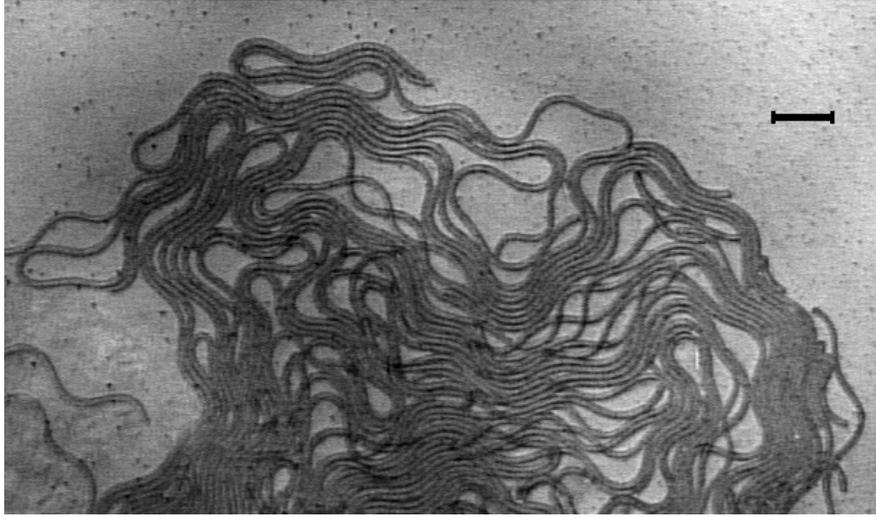


Fig. 4 Low magnification visible light microscope image of the frozen *Spirulina platensis* after rapid freezing. Scale bar is 40  $\mu\text{m}$ .

#### Bibliography:

1. Rippka, R. "Isolation and purification of cyanobacteria" in L. Packer, A. Glazer (Eds.) *Methods in Enzymology*. (Academic Press. San Diego. 1988). p 28
2. Catherine Hong and Susan Spiller. Maintaining a Laboratory Culture of *Spirulina subsalsa*, *Spirulina platensis* (*Arthrospira*, Rippka 1988) and three species of *Spirogyra* on agar plates and liquid medium or with sucrose or streptomycin. Nov. 2000-Barrett Undergraduate Research Reports. Department of Biology, Mills College, Oakland, California. (2000).
3. Carolina Biological Supply Company. P.O. Box 6010, Burlington, NC 27216-6010. carolina@carolina.com
4. The following method may be appropriate for examining these specimens. Gerd Schneider is visiting from Germany. One of his students will publish a doctoral thesis soon about x-ray, cryo-tomography of *Chlamydomonas reinhardtii* cells. He used a drawn out micro capillary as a cuvette/slide the frozen cells were imaged with 2.4 nm x-rays from various angles. The mathematics for 3-D imaging is worked out in the thesis. The samples have to be very small. Probably the *Spirulina subsalsa* will work if it can be drawn into the capillary as a filament.

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