Whole-Cell Biosynthesis of Nanostructured Metal Oxide Semiconductor Materials

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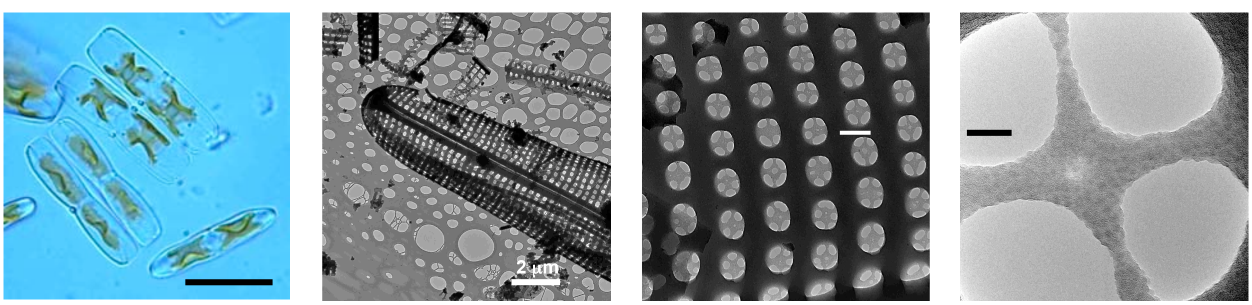
Overview. Nanostructured metal oxide semiconductor materials possess novel optical and electronic properties that can be tuned by manipulating the dimensions of the composite nanophases in the 1-100 nm size range. These materials have a host of potential applications, particularly for the fabrication of optoelectronic devices. However, it is extremely difficult to fabricate nanostructured semiconductor materials and at the same time integrate these materials into patterned device features that possess the nanoscale and microscale elements needed for the next generation of optoelectronic devices. Current technologies for fabrication of nanopatterned semiconductor materials are "top down", meaning that large and sophisticated equipment must be used to fabricate microscale and nanoscale features into the device structure.

In the first year of this project, we demonstrated that the bio-mineralization capacity of single-celled algae called diatoms (the *Bacillariophyceae*) can be harnessed to biologically fabricate nanostructured materials composed of silicon and germanium oxides. Furthermore, the nanostructured metal oxide materials are self-assembled by a "bottom up" approach into micron-scale structures called frustules, which possess submicron scale (100-1000 nm) features, such as periodic aperture arrays (*Figure 1*). These materials also possess nano-enabled optoelectronic properties, including strong blue photoluminescence. One could imagine these optoelectronic microstructures could serve as "breadboards" for the fabrication of microscale devices.

Bioprocess technology is used to metabolically insert foreign dopant metals (for example germanium) into the silica frustule of the living diatom cell and then produce copies of each nanocomposite frustule on a massively parallel scale, typically on the order of one million of these "nano-enabled microdevices" per milliliter of liquid cell culture suspension!



(a) Living cell. Scale bar 20 μm. **(b)** Metal oxide frustule. Scale bar 2 μm. **(c)** Periodic pore array. Scale bar 200 nm. **(d)** Nanostructures in pore. Scale bar 20 nm.

Figure 1. Micro-to-nanoscale features of the marine diatom *Pinnularia sp.*

Highlights of Research Results

Objective 1: Develop perfusion-based bioreactor cultivation strategies for the biosynthesis of nanostructured composite metal oxide materials, including oxides of silicon, germanium, and titanium, by diatom cell cultures (Rorrer Group). In Year 1, we developed a bioprocessing strategy where germanium was incorporated into the silica frustule of the diatom by a two-stage bioreactor cultivation process. In Stage 1 of the cultivation, the diatom cell suspension was grown on nutrient medium containing $Si(OH)_4$ (silicic acid) as the growth-limiting substrate within a bubble-column photobioreactor. Once all the soluble silicon was consumed, cell division ceased and the culture was silicon starved. In Stage 2 of the cultivation, a mixture of $Ge(OH)_4$ (germanic acid) and $Si(OH)_4$ dissolved in the liquid feed medium was added to silicon-starved cell culture. The diatom cells co-assimilated soluble silicon and germanium by a surge uptake mechanism, and then underwent one cell division or more cell divisions, depending upon the amount of silicon added. After one cell division, cells bearing a new frustule containing both silicon and germanium oxides were formed (*Figure 2*).

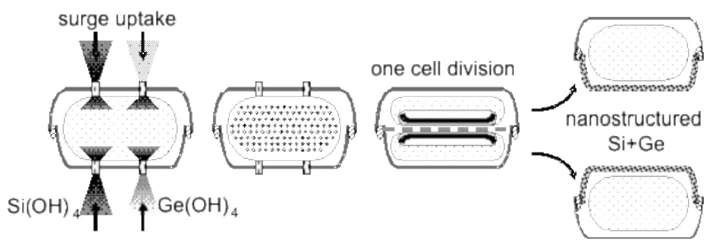
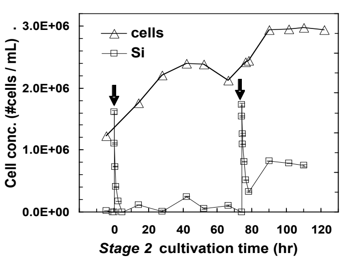
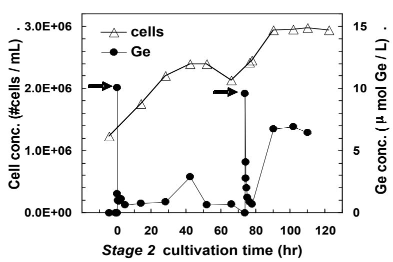
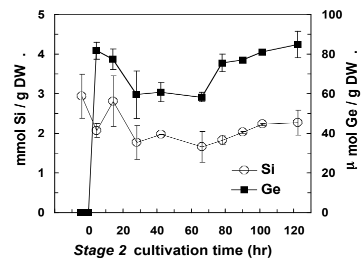


Figure 2. Two-stage bioreactor cultivation process for metabolic insertion of germanium into the frustule of *Pinnularia sp.* Arrows indicate points of co-addition of soluble Si+Ge feed solution.

(a) Model of Si & Ge uptake leading to nanocomposite metal oxides.

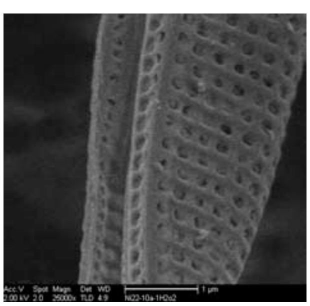




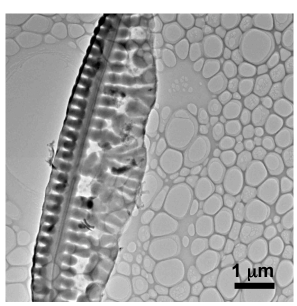


(b) Si consumption and cell division. **(c)** Uptake of Ge during Stage 2. **(d)** Si & Ge content in cell mass.

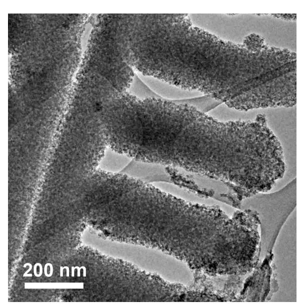
Objective 2: Characterize the chemical structure, nanostructure, and microstructure of the composite metal oxide materials fabricated within the diatom frustule (Rorrer, Chang, and Jiao Groups). The diatom cell mass from Stage 2 of the bioreactor cultivation experiment described under *Objective 1* was treated with aqueous hydrogen peroxide at ambient conditions to oxidize the organic materials away from the diatom frustule. The resulting amorphous metal oxide frustules were imaged and analyzed by SEM, TEM, and STEM-EDX.



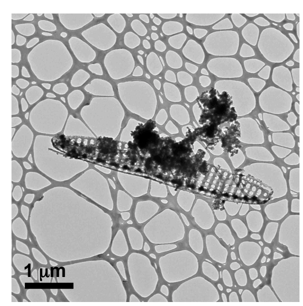
(a) *N. frustulum* before metabolic insertion of germanium.



(b) After metabolic insertion of germanium (0.6 wt% Ge).

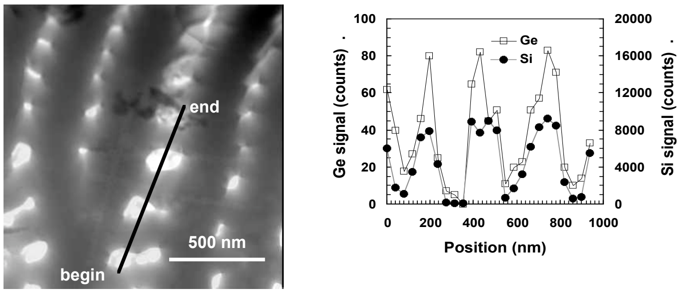


(c) Alteration in submicron pore structure array (0.6 wt% Ge).



(d) Frustule disintegration into nanoparticles (2.0 wt% Ge).

Figure 3. Alterations in submicron pore structure following metabolic insertion of germanium into frustule of *Nitzschia frustulum.*



(a) Line scan thorough aberrant pore array.

(b) EDX analysis of silicon and germanium.

Figure 4. Nanocomposite profile of *Pinnularia sp.* frustule after metabolic insertion of germanium.

In Year 1, we found that metabolic insertion of germanium into the diatom frustule during Stage 2 of the bioreactor cultivation experiment induced aberrations into the diatom frustule morphology at the submicron and nanoscales. The final nano- and microstructure of the frustule bearing the silicon and germanium oxides depended upon the amount of germanium and silicon added to the Stage 2 of the cultivation process, as shown in *Figure 3* for *Nitzschia frustulum*. Continued studies with *Pinnularia sp.* showed that metabolic insertion of germanium into the diatom frustule during Stage 2 of the bioreactor cultivation by the "single pulse" Si+Ge co-addition strategy resulted in uniform distribution of germanium and silicon within the frustule (*Figure 4*).

Objective 3: Characterize the optoelectronic properties of the nanostructured composite metal oxide materials, after the appropriate post-processing of the biogenic inorganic material (Rorrer, Chang, and Jiao Groups). Photoluminescence is a fundamental property of optoelectronic materials. Since photoluminescence is also a quantum mechanical phenomenon, it is often enabled and tuned by ordering the semiconductor materials at the nanoscale.

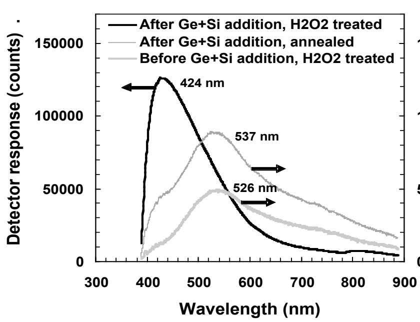
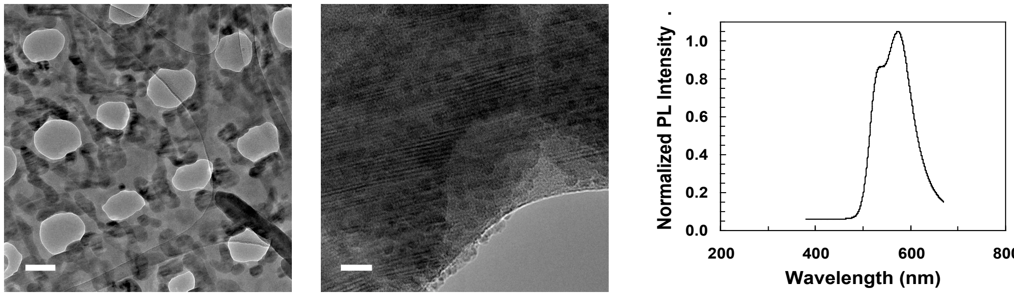


Figure 5. PL spectrum of metal oxide frustules derived from *Nitzschia frustulum* before and after metabolic insertion of germanium (0.6 wt% Ge in frustule).

Our results for Year 1 show that metabolic insertion of germanium into the diatom frustule during cell wall biosynthesis was needed to impart photoluminescent properties to the metal oxide frustules derived from the diatom cell mass (*Figure 5*). Furthermore, aqueous hydrogen peroxide treatment of the cell mass, which is a room temperature process for removing organic material from the metal oxide frustule, was also required to impart photoluminescent properties to this material (*Figure 5*). Thermal annealing quenched the photoluminescent properties of the biogenic silicon-germanium nanocomposite frustules. The fundamental reasons for these results are not known and will be investigated during years 2 to 4 of the project.

During Year 1, "post processing" efforts focused on making nanostructured zinc silicate composite materials with defined microstructure that possess photoluminescent properties. Using a chemical bath deposition (CBD) technique, a hydrothermal reaction step at 150 ^{o}C for one hour deposited a layer of Mn-doped zinc oxide onto the outer surface of the silica frustule of *Pinnularia sp.* Subsequent thermal annealing in air at 800 ^{o}C for 1.0 hr converted the zinc oxide to nanocrystalline zinc silicate (*Figures 6a,b*). The nanocrystals did not fill the submicron pores of the frustule and hence did not alter the microstructure. This material possessed intense green photoluminescence centered at 575 nm (*Figure 6c*).



(a) Zinc silicate coating. Scale bar 100 nm.
(b) Imbedded nanocrystals. Scale bar 10 nm.
(c) PL spectrum after thermal annealing.

Figure 6. Nanostructured coating of zinc silicate on *Pinnularia sp.* frustule.

Education and Outreach. During the first year of this grant, the five graduate students assigned to this project were cross-trained in the following areas: bioprocessing and cell culture; electron microscopy; and optoelectronic materials characterization.

This grant has a unique outreach effort called the "*Adventures of Frusty & Cyclo*" project (website: http://che.oregonstate.edu/research/ndevan/Index-10.html). "Frusty & Cyclo" are anthropomorphic cartoon characters created by a team of middle school girls based upon two marine diatoms, *Nitzschia frustulum* and *Cyclotella sp.* (*Figure 7*). Each "Adventure" is a comic strip which communicates a basic scientific message relevant to biotechnology and nanoscience. On September 24th, 2005, the "Adventures of Frusty & Cyclo" project had a booth at the Corvallis Fall Festival, a prominent art show in the Pacific Northwest. This booth sold hand-made inexpensive crafts made by the middle school girls based upon the Frusty & Cyclo characters, such as "Frusty Earrings" consisting of "nanoparticle" beads. A pamphlet describing the research project, written in layman terms by the middle-school girls, was handled out to each person who stopped by the booth. A total of 79 pamphlets were distributed.

Research Activities supported by this grant have also received exposure by popular media. In the first year of this grant, the research was highlighted on *National Public Radio*, *National Geographic News*, and several websites.

(a) Frusty & Cyclo.
(b) Reading the pamphlet.
(c) The team.

Figure 7. The "Adventures of Frusty & Cyclo" at Corvallis Fall Festival.

References

[1] For further information about this project, please email Greg Rorrer at rorrergl@engr.orst.edu.