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The Diatoms of Antarctica and their Potential Roles in Nanotechnology

by Richard Gordon, Andrzej Witkowski, Ille Christine Gebeshuber & Claire S. Allen

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Richard Gordon, a theoretical biologist, got hooked on diatoms because of the fascinating movement of the colonial diatom *Bacillaria paradoxa*. He has tried to understand the motility mechanism and the morphogenesis of the diatom shell, meanwhile inadvertently starting the field of diatom nanotechnology. His other research includes morphogenesis of early vertebrate embryos, detection of early breast cancer, and halting of the HIV/AIDS epidemic.

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Andrzej Witkowski is a geologist and oceanographer by training who got involved in diatom studies while working on his Ph.D. on fossilization processes of siliciclastic microbial mats. Since then his research has focused on paleo aspects of marine diatoms and species composition, biodiversity and biogeography of marine littoral diatoms worldwide. Recently he has investigated marine littoral diatoms of the South African Kerguelen Islands and some parts of Antarctica. He is Editor of Diatom Monographs.

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Ille C. Gebeshuber is an experimental physicist who has been working with diatoms since 1999 when she was the first to image live diatoms *in situ* under water with nanometer resolution and found self-repair properties of diatom adhesives, which make them interesting for man-made high-tech underwater glues. In 2004 she founded the field of diatom tribology, where diatoms are used as inspiration for the development of man-made micro- and nanoelectromechanical machines (MEMS and NEMS). The amazing click-stop mechanism in the Antarctic diatoms *Corethron pennatum* and *C. criophilum* inspires us to build the tiniest optimized MEMS structures that unfold and are then irreversibly fixed.

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Claire Allen is a palaeoceanographer at the British Antarctic Survey, currently working within the 'Quaternary Sediments' work package of the 'Chemistry and Past Climate' research programme. She uses diatoms preserved in marine sediments to reconstruct past changes in the ocean and climate of Antarctica to better understand Antarctica's role in the global climate system. Claire has participated in six Antarctic science cruises throughout the Antarctic Peninsula and Scotia Sea, collecting water and sediment samples to help constrain the ecological significance of Southern Ocean diatom records.

Biological nanotechnology and man-made nanotechnology currently still differ substantially (Abdel-aal & Ille C. Gebeshuber, 2010). Whereas in biological systems, intricate hierarchical structures are built with integrated functionalities on various length scales (from the nanometer scale to the micrometer scale to the millimeter scale), engineers still struggle to control shape or functionalities in their nanomachinery; and with the development of hierarchical technical structures we are just at humble beginnings. Diatoms, with their amazing structures and functionalities, with their ability for cell-division once a day or even more often, and with their ability to produce silica even in Antarctica, where the water temperature is below the freezing point, can serve as inspirations in human technology, yielding sustainable materials, structures and processes. Diatoms have become fascinating to the industrial world over the past two decades primarily because of their ability to create solid structures of many types at a wide range of size scales. Some of them have motors with no moving parts that run at 99.9% efficiency (Gordon, 1987), and all produce oil from which we could run our cars in a sustainable way (Ramachandra et al., 2009). Antarctic diatoms accomplish these feats under conditions that are extreme, such as low temperature, at high salinity embedded in or attached to ice (Janech et al., 2006; Krell et al., 2008), and often under high ultraviolet radiation from the sun (Helbling et al., 1996; Skerratt et al., 1998; Hernando & Ferreyra, 2005; Wulff et al., 2008). They survive the Antarctic winter for 6 months with little or no light (Wulff et al., 2008) and many remain viable even when frozen into sea ice or buried in sediment, with some able to germinate after many years (Davis, 1972; Zgurovskaya, 1977; Hollibaugh, Seibert & Thomas, 1981; Ligowski, Godlewski & Łukowski, 1992). Antarctic diatoms thus rightfully belong to those microorganisms that are called extremophiles (Sterrenburg et al., 2007). As we shall show, these properties may prove useful in applications of diatom nanotechnology.

Diatoms are both the cause and possible solution to the present global warming crisis, though global warming has at least one positive side, as farming has contributed to it for 5000 years and may have postponed the next ice age (Ruddiman, 2005). Those of us who put up with or escape Canadian winters sometimes schizophrenically yearn for global warming. The role of diatoms is quite simple: much of the crude oil that we take out of the ground comes from diatoms, and can still be seen within the shells of diatoms long dead (Figure 1). If we were to switch to live diatoms, perhaps living in solar panels and genetically engineered to secrete their oil, or even gasoline itself (Ramachandra et al., 2009), then there would be no net increase in carbon dioxide: they would take up as much as we put into the atmosphere on burning their oil. Furthermore, there would be no need to switch to new kinds of automobile engines, such as electric or hydrogen

based.

Nanotechnology has come to mean all things tiny that we manufacture. One Nobel Prize winning physicist, Robert B. Laughlin, calls this infatuation the production of “*nanobaubles*, fascinating and beautiful structures that develop spontaneously at small scales but have no known use except as entertainment.... While our knowledge of the nanoscale is exploding almost incomprehensively at the moment, nearly all of it is deeply unimportant.... The idea that nanoscale objects ought to be controllable is so compelling it blinds a person to the overwhelming evidence that they cannot be” (Laughlin, 2005).

The ordinary approach to nanotechnology is to create it by lithography (Sargent, 2005; Dupas, Houdy & Lahmani, 2007; Reisner, 2008; Gebeshuber, 2009) or chemical reaction, often biochemical (Goodsell, 2004; Rehm, 2006; Renugopalakrishnan & Lewis, 2006; Gazit, 2007; Papazoglou & Parthasarathy, 2007). Here our focus is on diatom nanotechnology, in which one uses living organisms to grow desired nanostructures rather than trying to build them ourselves:

“Diatoms... generate nanostructured silica microshells (frustules) with thousands of species-specific morphologies. Sustained reproduction of a particular diatom species can yield enormous numbers of frustules with similar 3D morphologies. Such intricate, genetically precise, and massively parallel 3D self-assembly under ambient conditions lies well beyond the current capabilities of synthetic micro- and nanofabrication” (Weatherspoon et al., 2007).

In an offhand way, one of us started the field of diatom nanotechnology when invited to give his first talk at an engineering conference, and not knowing what to say, decided to suggest that diatoms could make things engineers wanted (Gordon & Aguda, 1988). It has since burgeoned into a large effort by many people, tying together biologists, called diatomists, with industrialists (Gordon, Sterrenburg & Sandhage, 2005; Kroth et al., 2007; Allison et al., 2008; Kröger & Poulsen, 2008; Bozarth, Maier & Zauner, 2009). The plans are wide sweeping, including “everything but the kitchen sink” in miniature form:

“Actuator, antenna, bar, bearing, cantilever, capsule, catalyst, cone, cube, cylinder, die, diffraction grating, disk, fiber, filler, filter, funnel, gear, heat exchanger, hinge, honeycomb, insulator, lens, lever, light pipe, magnet, membrane, mesh, mirror, mixer, motor, needle, nozzle, piston engine, plate, prism, pulley, pump, reactor, refraction grating, relay, rocket, rotor, sensor, separator, sieve, sphere, spiral, spring, substrate, switch, syringe, tag, tetrahedron, tetrakaidecahedron, transducer, tube, turbine engine, valve, wedge, wheel”

(Gordon, 2010).

One might agree with Laughlin, because this perhaps \$100 million investment so far has yet to yield a single commercial product (Gordon, 2010). But perhaps in time it will. Certainly, diatoms figure in an art form little appreciated by the art world: the careful arrangement of diatoms to produce patterns and pictures only visible with a microscope (Nagy, 2002; Matthias Burba, 2008; Kemp, 2009) (Figure 2). Diatoms, including those from Antarctica, have appeared on postage stamps (Figure 3).

Diatom nanotechnology is intimately tied to the field of morphogenesis, in which one tries to figure out how organisms get their shapes (i.e., morphology). This is one of the basic, unsolved problems of biology. One of the refreshing aspects of all this attention to diatoms is that, in order to manipulate them, most industrialists and their scientist collaborators believe it would help to figure out how diatoms actually create their silica structures. Thus basic science is getting an enormous boost, and many pieces of the puzzle have been found, if not yet put together into a coherent mechanism (Gordon, 2008). So playing with all these nanobaubles just might lead to breakthroughs in our understanding of life in general: “Lots of money is sloshing around, and great fortunes are being made and lost.... The allure of traveling in such a wild and lawless place is the ever-present possibility of making a serendipitous discovery of great importance” (Laughlin, 2005).

The basics of diatom morphology have already been covered in this book (Scharek, 2010). Diatom assemblages of the Antarctic and the Austral Islands are unique in many respects. Firstly they inhabit either sea ice or cold to very cold waters (Medlin & Priddle, 1990). Secondly their habitats are subject to very strong winds. The latter phenomenon results in mixing of the assemblages. Hence marine and likewise terrestrial assemblages are composed of both marine and freshwater forms (Witkowski, Riaux-Gobin & Daniszewska-Kowalczyk, 2010). In addition the whole area determined as Southern Ocean is isolated from surrounding areas by a strong hydrologic barrier – the Polar Front. The existence of the Polar Front significantly reduces the exchange of the organisms with the other more northern geographic regions. Under such harsh environmental conditions peculiar diatom assemblages have developed composed of diatom species that are endemic for the Antarctic (Southern Ocean) as outlined by the Polar Front. Included in this assemblage are numerous genera, but the most interesting are *Fragilariopsis* and *Eucampia*. They are indicative either for marine ice or for conditions in the water column. Due to this they also play very important role as indicators of climate change (Zielinski & Gersonde, 1997). In our Figures 5-6 examples of taxa

representing *Fragilariopsis* and *Eucampia* are shown, all endemic for the Southern Ocean (Hasle & Syvertsen, 1996).

The diatom assemblages we studied originated from fairly deep stations (up to 50 m). They were high in species number and predominantly composed of benthic species, though planktonic and ice diatoms were also observed. This is only possible due to very high transparency of the water column. So far little is known about ecology and species composition of Antarctic littoral/sublittoral (near shore) diatom assemblages. Research effort is mainly focused on sea ice and planktonic diatoms as they play important role in primary production of the Antarctic waters. Hence numerous studies were focused on survival strategies of Antarctic diatoms. Interestingly some of the planktonic diatoms form wintering stages (Fryxell, 1994) while others survive in sea ice (Cunningham & Leventer, 1998).

This imbalance in attention to sea ice and planktonic diatoms on one hand and marine benthic diatoms of the Antarctic on the other is difficult to understand as the latter forms offer much more diverse and novel morphologies, which are possible to observe nowhere else in the world's ocean (Figures 5-6). This provides exciting research opportunities first of all for diatomologists dealing with biodiversity and taxonomy (new species and higher taxa) and to nanotechnologists (new ultrastructures).

Let's now have a look at some diatom parts, to see why engineers are intrigued with their potential. We'll use examples from our own work. A simple one is the circle or hoop, which comes from the silica girdle bands found around most diatoms. What is remarkable in this example is the high degree of perfection of circularity, as if it were "precisely machined" (Figure 7), but we have no lathes that small. Of course, a ring is but a component in a potential nanomachine, but that is the current state of the art: no one has assembled any device yet with moving diatom parts. A nanorobot (nanobot) consisting of a diatom shell propelled by bacteria motors has been proposed for carrying drugs inside our bloodstream (Figure 8), and one might imagine the optical diffracting powers of diatom shells to be put to work in fancier arrays than movable micromirrors (Link & Zimmerman, 2007).

Diatom morphologies are amazing not only because of their geometric variety and perfection but also because of their size range. Antarctic diatoms range in size from just 1-2 microns in length and width (e.g. *Fragilariopsis cylindrus*) to 3-4 mm in length and only a few microns wide (*Thalassiothrix* spp., *Trichotoxon* spp. and *Entopyla* spp.) and up to several hundred microns diameter

(*Arachnoidiscus* spp. and *Coscinodiscus* spp.). Even within a single species the size ratio of individuals can be almost an order of magnitude (10x)! This may be especially true in Antarctica where the extreme conditions may inhibit sexual reproduction except perhaps for once a year in a massive orgy (Crawford, 1995; Crawford, Hinz & Rynearson, 1997).

Claire Allen 1/8/10 4:44 PM

Comment: Is this likely or are the number of cell divisions between sexual cycles 'fixed'?

Figure 9 shows some of the largest diatoms found in Antarctic waters and exemplifies their different shapes and ornamentation. In Antarctica, diatom distribution is affected most profoundly by the seasonal progression of sea ice. This is especially true for large diatoms, because pore spaces within the ice are small and diatoms larger than approx. 30-40 microns are easily crushed in the freezing process. As such, the largest diatoms in Antarctica must survive beneath the sea ice - deep in the water column, in benthic and epibenthic habitats, or in the permanently open ocean beyond the sea-ice. With the exception of *Coscinodiscus asteromphalus*, the diatoms pictured in Figure 9 are all benthic and epibenthic diatoms. The 'giant' benthics also seem to be more cosmopolitan than the planktonic diatoms, with many occupying littoral regions in temperate and tropical latitudes as well as the icy coasts of Antarctica. *Arachnoidiscus japonicus* (Figure 9a) is more commonly found in the tropical Pacific and has only recently been found in Antarctica (Al-Handal & Wulff, 2008). Its size range in Antarctica appears to exceed descriptions elsewhere and may reflect an algal version of the 'gigantism' seen in other Antarctic marine organisms (Woods et al., 2009)!

Claire Allen 1/8/10 4:44 PM

Comment: Not newly arrived just newly discovered!

The common Antarctic diatoms species *Corethron criophilum* and *C. pennatum* are exquisite examples for integrated mechanics on the micro- and nanoscale (Figures 10-12). Their structure and function is of high interest to nanotechnologists (Gebeshuber & Crawford, 2006; Gebeshuber, 2009). *C. criophilum* and *C. pennatum* exist as single cells (i.e., they do not form colonies) with two different valves per cell. One hemispheric valve has a set of long spines that are attached to the valve at a series of sockets on the rim of the valve (Figure 10 right). The other valve has similar spines, but alternating with them is a series of finer hooked spines (Figure 10 left). The spines can move to a degree in the socket, but the position in which they are found in the mature, independent cell is not where they are formed. The process of new valve formation in these diatoms is complicated (Crawford & Hinz, 1995; Crawford, Hinz & Honeywill, 1998). The spines are formed along with the new valves, within, and protected by the cylinder of the two sets of girdle bands (Figure 11). During the cell division cycle, the cell elongates greatly, forming very many girdle bands as it does so, thus creating a long space between the two sibling cells when they are complete. This space can accommodate the formation of the long spines. When the new cells are mature, they expand and pull

the girdle cylinder away from the base of the spines and allow the spines to swing out to adopt their final position (Figure 12). In doing this, they move past a click-stop that prevents them moving too far back from their ‘required’ position. This unique case of a click-stop mechanism in rigid micromechanical parts is a ‘best practice’ example for the connection of structure with function in nature.

The Antarctic diatom *Corethron* serves as inspiration for micro- and nanotechnology, where it is of paramount importance to come up with novel ideas on how to fabricate three-dimensional structures from two-dimensional structures, by unfolding and subsequently fixing them (Gebeshuber & Crawford, 2006; Gebeshuber et al., 2009). The question that remains to be answered by future diatom researchers is: What causes the long spines to pull the girdle cylinder away and to move past the click-stop point? Imagine flat *Corethron*-inspired MEMS and NEMS that would unfold, expand and fix themselves in their predetermined final three-dimensional structure on demand!

One common characteristic of diatom shells is their fairly uniform pores (Figures 13-14). One potential use for these is to act as a selective filter, screening out larger molecules and letting smaller ones through (Figure 15).

Diatoms, due to their intricate structure at many size scales, have a huge surface area for their size. This makes them sensitive to tiny amounts of adsorbed gas molecules. Since their optical properties change when they adsorb gases, some experimental detectors of low levels of dangerous gases have been made from them (Lettieri et al., 2008). Various molecules can be bound to the surface of diatom shells, where they can react with other molecules and for a whole “lab on a diatom” (De Stefano et al., 2009).

One of the most advanced applications of diatoms is to turn their silica shells into other substances without changing their shapes. This is done by cooking them at high temperatures in a vapor containing atoms other than silicon. In this situation, when a silicon atom vaporizes from the shell, it is rapidly replaced by the alternative atom, so that the whole structure remains intact, not quite the Star Trek Replicator, but getting close (Drum & Gordon, 2003). The process is analogous to altering a brick building one brick at a time, replacing, say, each clay brick by a granite brick. The forces between adjacent atoms provide the “mortar”. With this approach, ceramic (Dickerson et al., 2005) and metallic alloy (Sandhage & Bao, 2008) “diatoms” have been made. Silica is $\text{Si}(\text{OH})_4$, and a similar process removes the oxygen and hydrogen atoms, leaving pure silicon (Si). An all silicon “diatom” can possibly be used to create new three dimensional computers (Bao et al., 2007). Diatoms can also be used as molds to make nanostructures of other substances

(Losic et al., 2007).

Genetic engineering of diatoms has already started with the complete sequencing of DNA in a few species (Genome Project, 2009c, b, a; Karthick, 2009) and direct manipulation of the genome (Kroth, 2007; Gordon et al., 2009).

The inherent bias towards benthic habitats means that Antarctica's giant diatoms are not hindered by heavy silicification and as such are far more robust than their tropical counterparts (eg. *Ethmodiscus* spp.). Whether or not large, heavily silicified shells affect oil production by the cells is not yet known, though large Antarctic diatoms may play a role in global cooling through carbon sequestration (Pollock, 1997), to which they significantly contribute (DiTullio et al., 2000; Grigorov, Pearce & Kemp, 2002). Let's then return to the problem of a sustainable source of gasoline, which might be based on Antarctic diatoms.

The USA leads in gasoline consumption with an average of 10 barrels per person per year (StateMaster.com, 2009). Suppose we could genetically engineer giant Antarctic diatoms to secrete say 25% of their volume in oil per day, while living inside specially designed solar panels (Ramachandra et al., 2009). The largest Antarctic diatoms are nearly all epibenthic ones and the largest centric is *Arachnoidiscus* spp. (typically 0.4 to 1 mm). The volume of one giant 2 millimeter wide diameter subtropical and tropical species *Ethmodiscus rex* or *E. gazellae* cell is about 4 cubic millimeters (Villareal et al., 2007), so a big Antarctic *Arachnoidiscus* would about 1 cubic millimeter. Ten barrels converts to 1.64 billion cubic millimeters. This volume of oil could then possibly be produced by 20 million cells in the course of a year. A double layer of 1 million *Arachnoidiscus* cells of 1 mm diameter would occupy 10 square meters. While these cells have large vacuoles occupying perhaps 99% of their volume (Woods & Villareal, 2008), other diatoms do not and can have up to 85% of their volume as oils or lipids (Ramachandra et al., 2009). Furthermore, we might be able to shrink vacuole sizes by deliberate selection (Gordon, 1996). This then gives a rough calculation of how much area would need to be covered by gasoline secreting solar panels for each person. The production per cell might be less, but the number of layers of cells could be more, and there is a wide range of size of cells and approximately 100,000 species of diatoms (Fourtanier & Kociolek, 2009) to choose from, so if we could get diatom secretion of their oil to work, it might be practical. The advantages of solar panels are that they may be placed on roof tops, walls, deserts, and other generally unproductive areas, don't compete with farming for food, and may be widely distributed, minimizing transportation costs to deliver gasoline.

In summary, diatom nanotechnology may prove to be both a profitable and fundamental endeavor. Antarctic diatoms (WynnWilliams, 1996), including those in inland lakes (Jones, 1996; Laybourn-Parry & Pearce, 2007), coming from a unique environment that is high in biodiversity (Brandt, 2005), and may be well worth our increased attention.

Figure 1. This is a 10,000 year old fossil diatom still containing chlorophyll, fluorescing red, and oil, fluorescing green. From (Stasiuk & Sanei, 2001) with permission [to be requested].

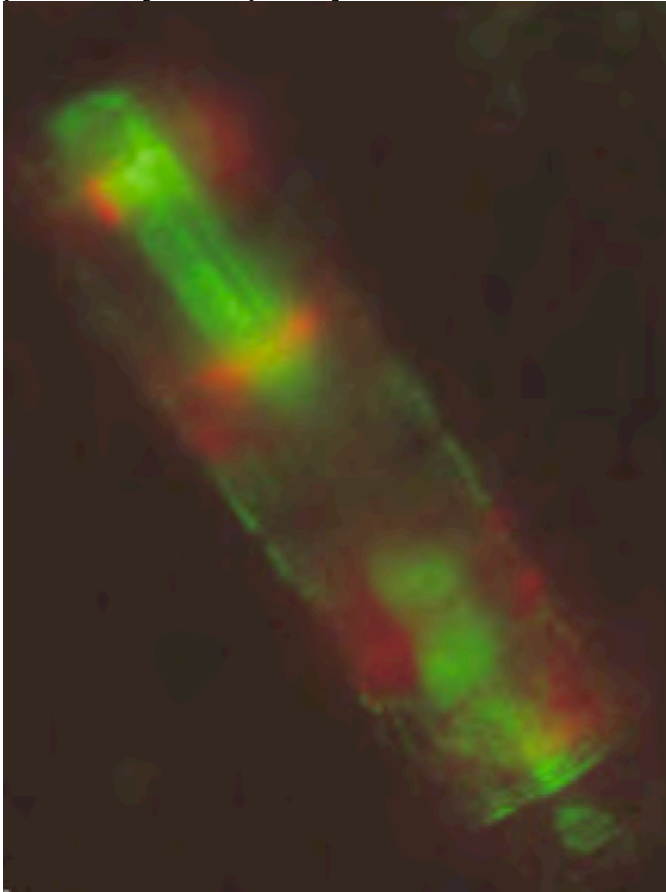


Figure 2. A diatom arrangement by Stephen S. Nagy, Montana Diatoms, with permission [to be requested].



Figure 3. All known examples of diatoms on postage stamps, including from Antarctica (Edlund, 2009), with permission [to be requested].



Figure 4. The Antarctic diatom *Eucampia antarctica* shown in scanning electron micrographs. Note that the bar size is in microns, with 1 micron = 1/1000 of a millimeter.

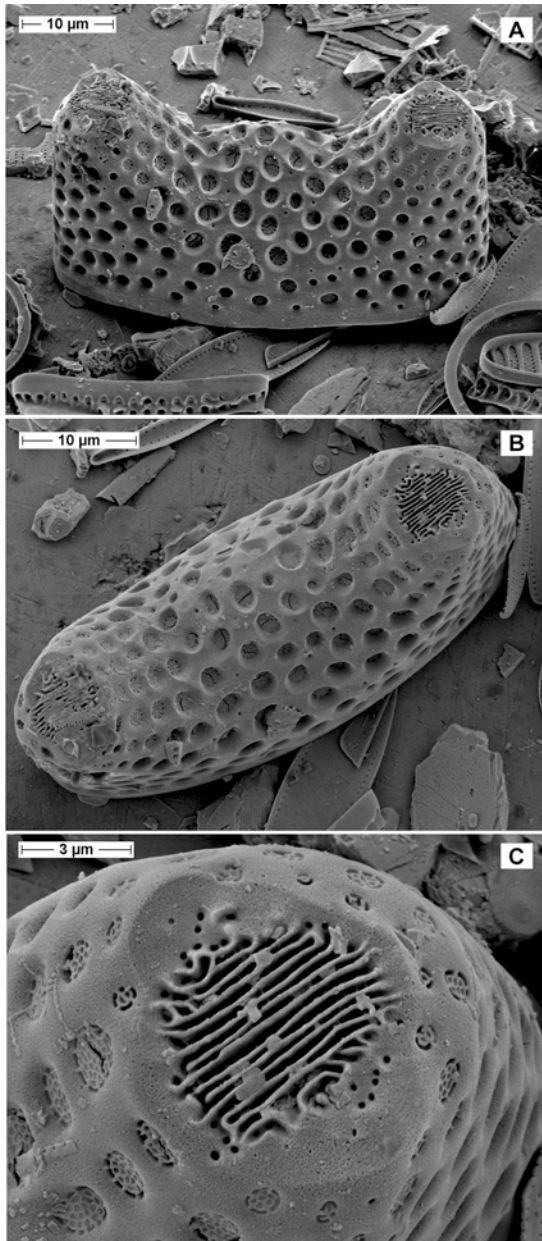


Figure 5. The Antarctic diatom *Fragilariopsis curta* (A-D) and *F. sublineata* (E) shown in scanning electron micrographs.

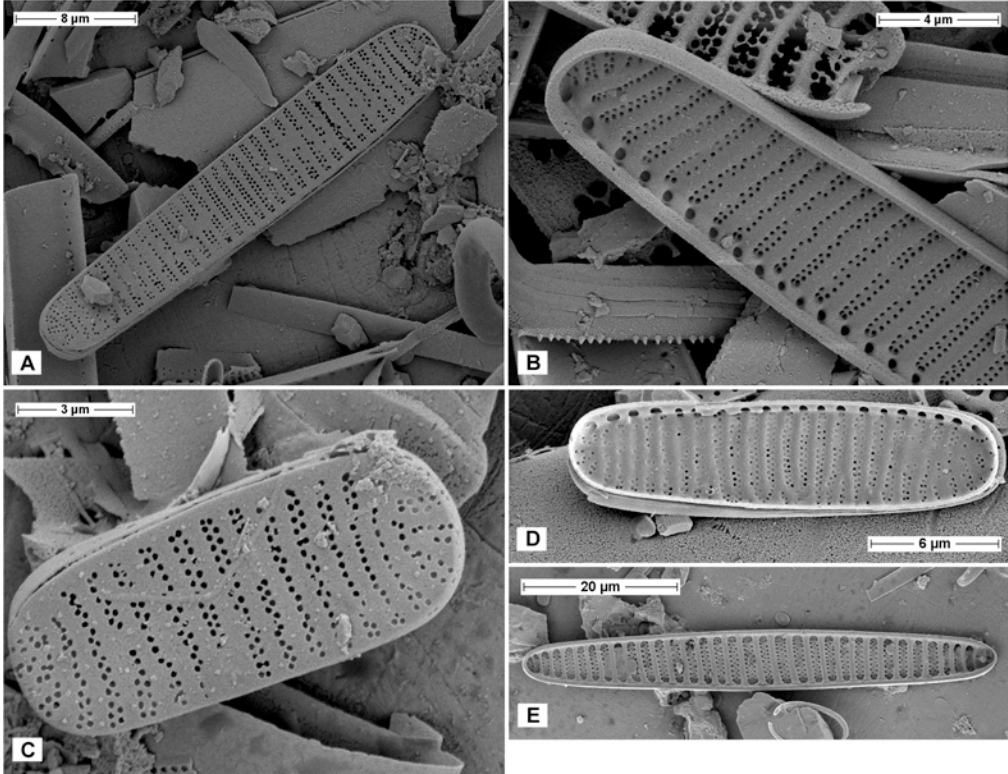


Figure 6. Light micrographs of some Antarctic diatoms. A-C: *Fragilariopsis kergulensis*. D: *F. angulata*. E, F: *F. separanda*. G: *F. curta*. H: *F. peragallii*. I, J: *F. ritscheri*. K, L: *Eucampia antarctica*.

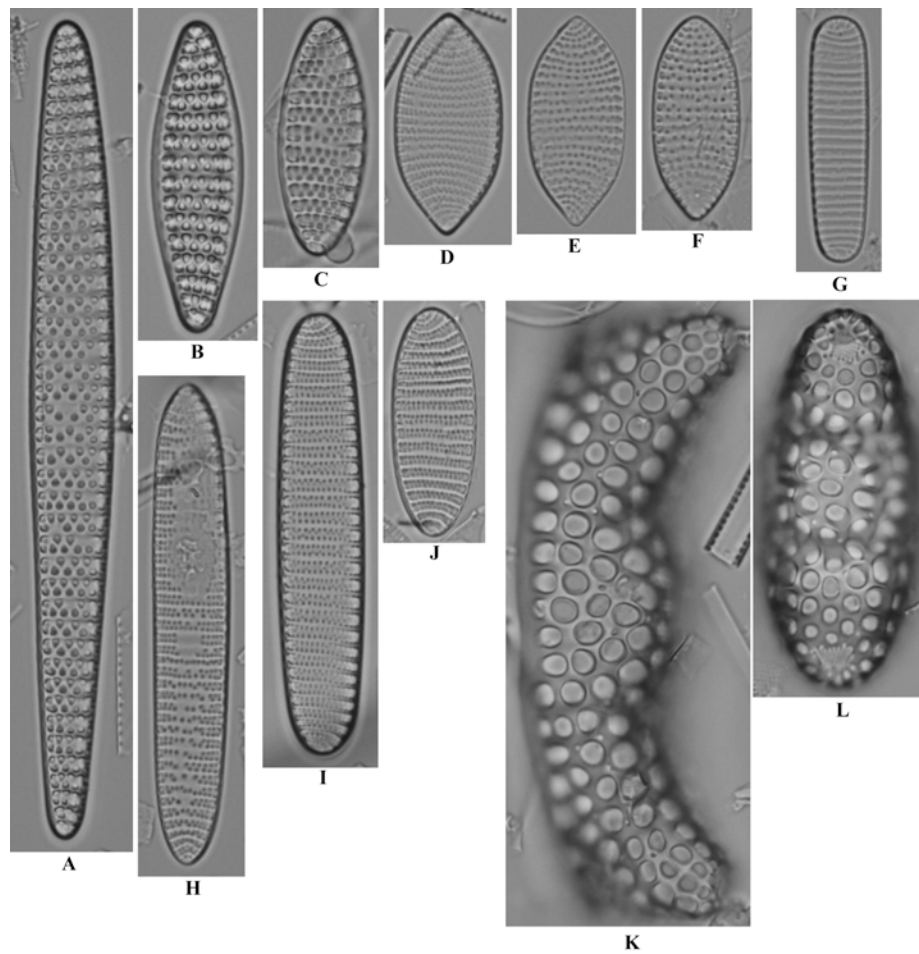
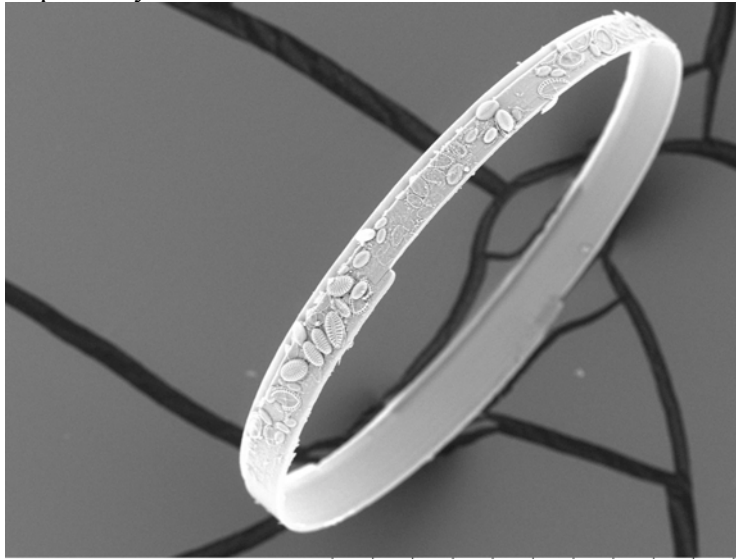
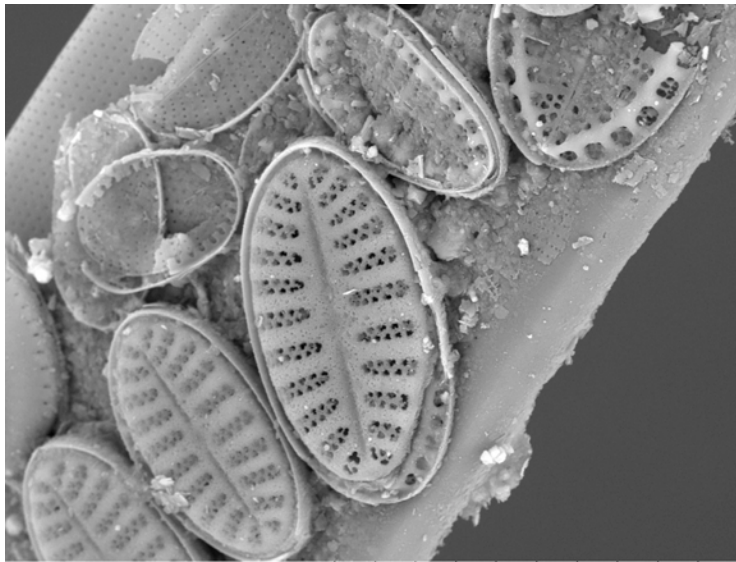


Figure 7. A girdle band 0.4 mm in diameter from a “giant” centric Antarctic diatom, *Arachnoidiscus* sp. It is decorated with much smaller, adhering *Cocconeis* pennate diatoms shown in the closeup. Scale bars are 0.3 mm and 0.03 mm, respectively.



TM-1000_0006 2008/12/08 L D2.0 x300 300 um



TM-1000_0005 2008/12/08 L D2.0 x3.0k 30 um

Figure 8. “Diatoms for drug delivery. Panel (a) shows exemplary SEM [scanning electron microscope] images of purified diatoms with whole, fraction-free frustules in comparison with raw diatomaceous earth (inset image). Panel (b) shows a multifunctional diatom-based drug-delivery system (i) and a model of a self-propelled drug carrier with diatom and attached bacterial biomotors (ii).” From (Gordon et al., 2009) with permission [to be requested].

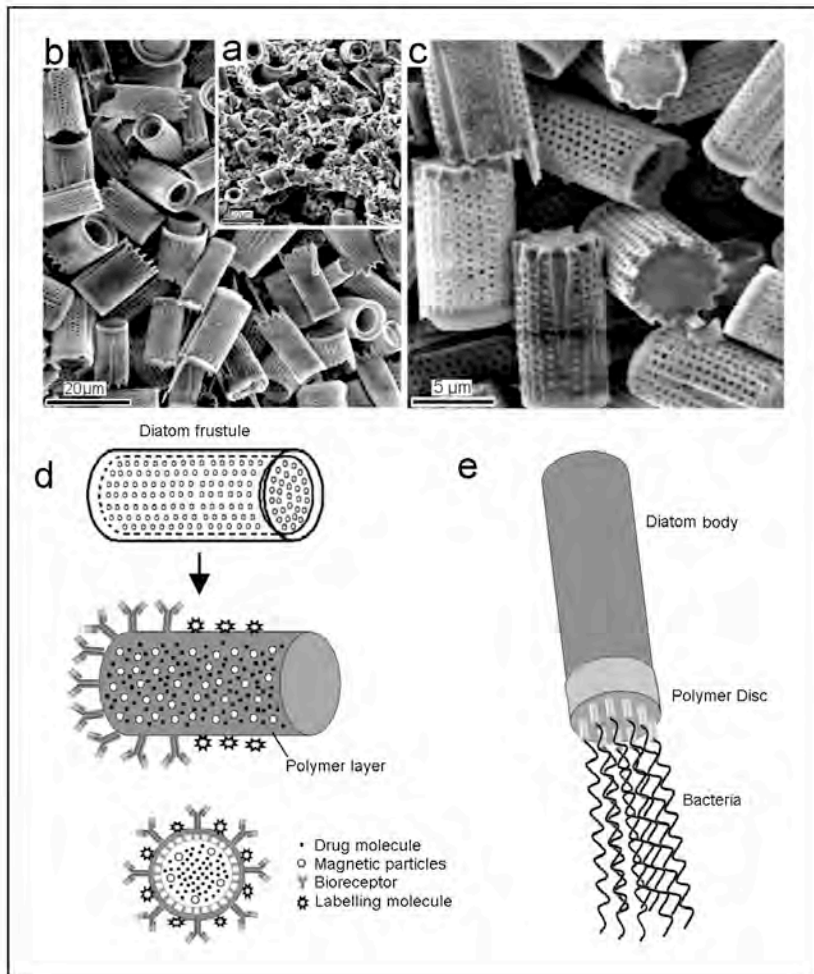
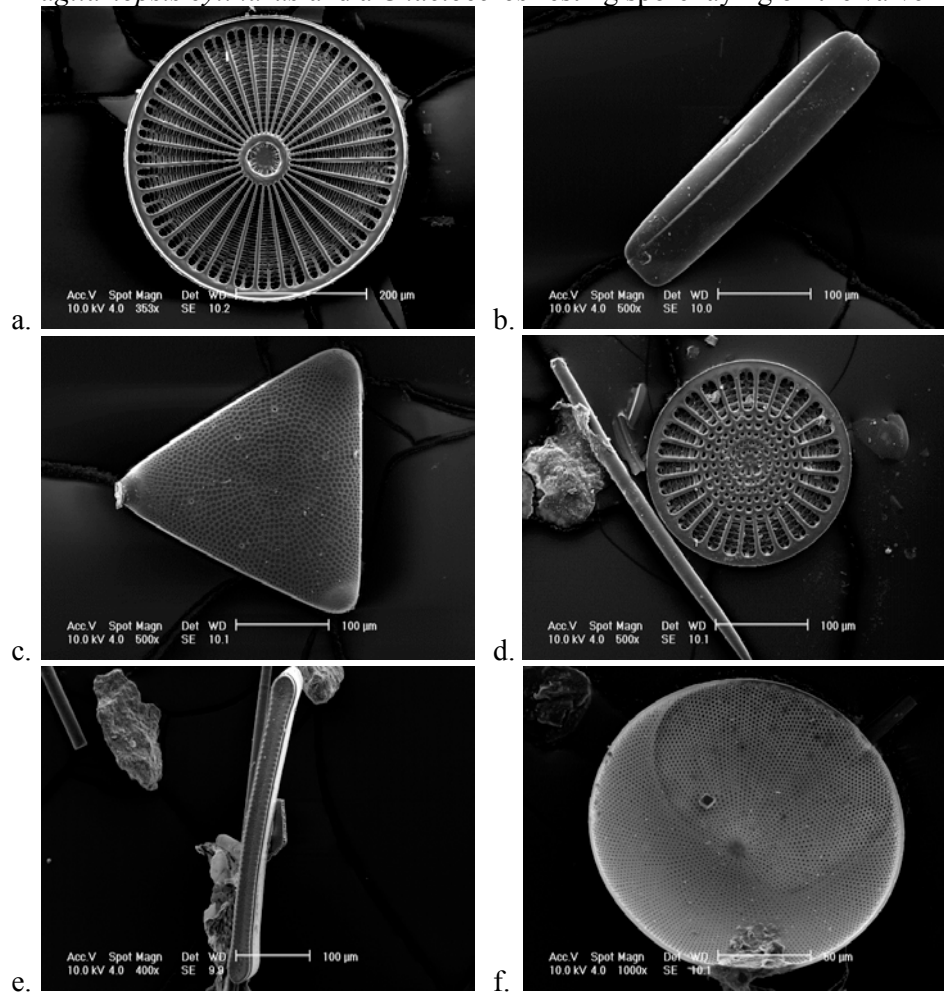
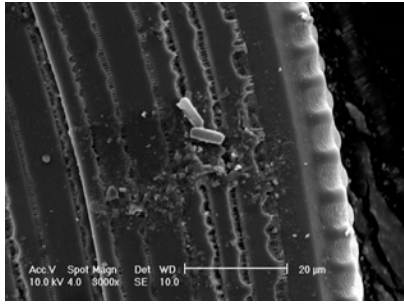
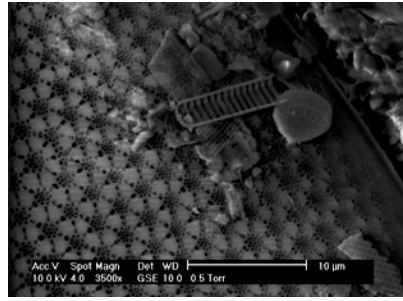


Figure 9. Giant Antarctic diatoms: a) *Arachnoidiscus* sp.; b) *Trachyneis aspera*; c) *Trigonium arcticum*; d) *Arachnoidiscus* sp.; e) *Entopyla* sp.; f) *Coscinodiscus asteromphalus*; g) close up of *Entopyla* valve with small *Fragilariopsis* sp. valves resting on the surface; h) close up of *Coscinodiscus* areolae pattern with *Fragilariopsis cylindrus* and a *Chaetoceros* resting spore laying on the valve face.





g.



h.

Figure 10. The two valves of *Corethron* at either end of a long cylinder of girdle bands. The valve to the right has a set of long spines attached to the rim, the valve to the left has similar spines, but alternating with them is a series of finer hooked spines. Scale bar 50 μm . Image used with permission [requested], © R.M. Crawford and F. Hinz, from (Gebeshuber & Crawford, 2006).

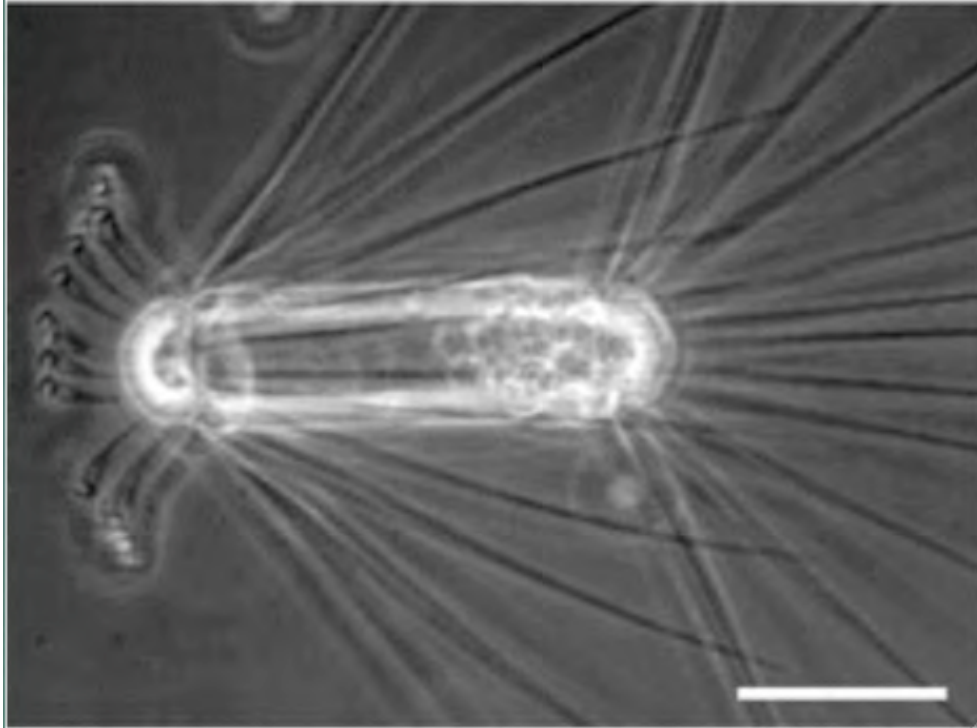


Figure 11. A cell of *Corethron* with the old valve (V1) to the left showing the base of a number of long spines and the new valve (V2) to the right. In the new valve, the spines are still oriented parallel to the cylinder of the girdle-bands. Scale bar 20 μm . Image used with permission [requested], © R.M. Crawford and F. Hinz, from (Gebeshuber & Crawford, 2006).

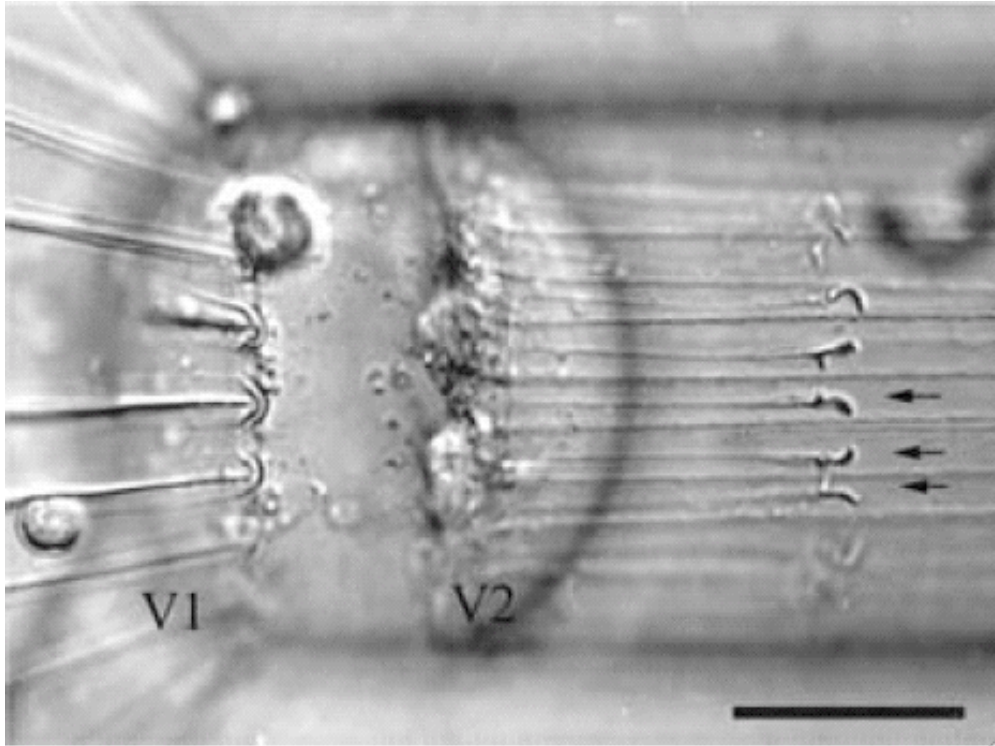


Figure 12. Surface view of a valve similar to that in the right in Figure 10, showing insertion of the long spines at the edge of the valve. Scale bar 10 μm . Image used with permission [requested], © R.M. Crawford and F. Hinz, from (Gebeshuber & Crawford, 2006).

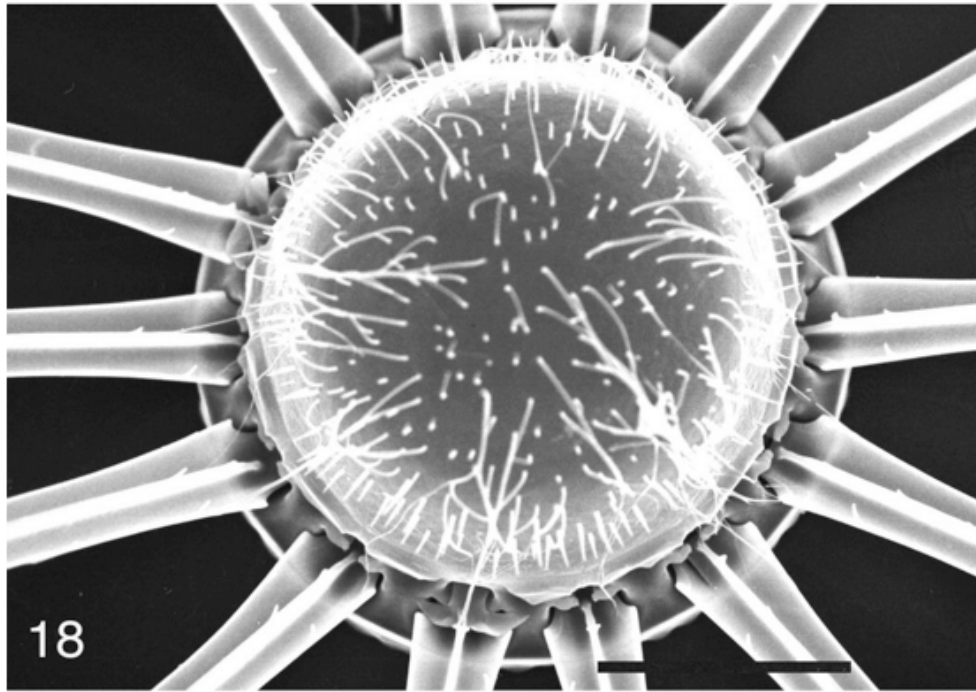


Figure 13. The valve view of a giant centric diatom *Arachnoidiscus* sp. 0.4 mm in diameter, found in Antarctic waters, showing an array of pores, which could be used for a nanofilter.

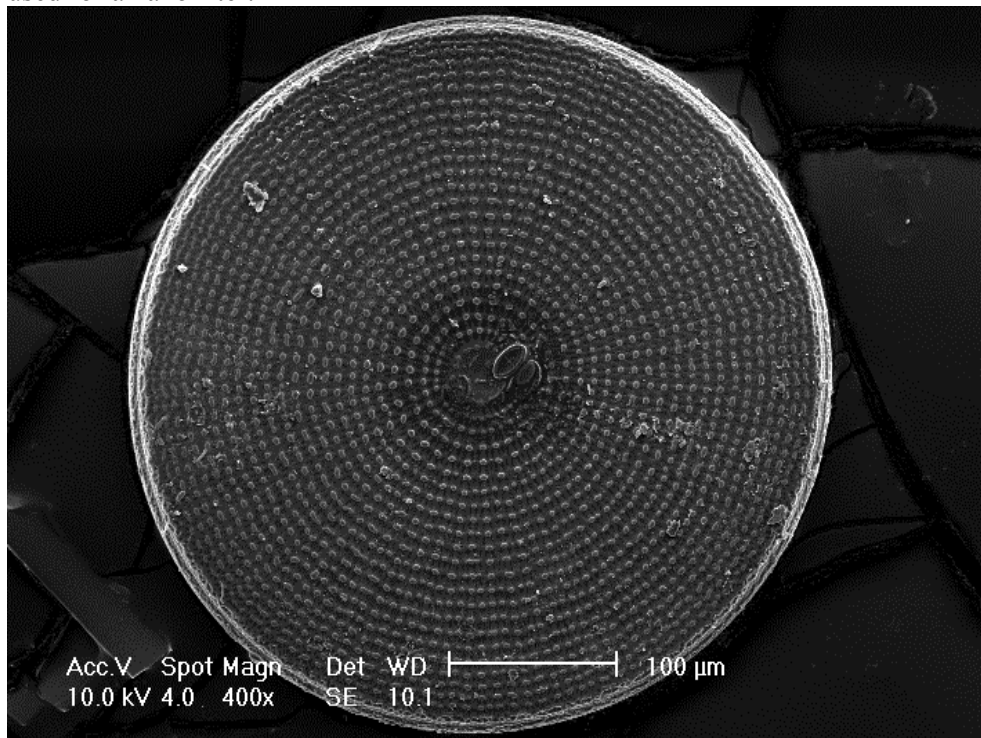


Figure 14. The valve view of a giant diatom *Trigonium arcticum* 0.5 mm wide, and a *Coscinodiscus asteromphalus* 0.25 mm wide, found in Antarctic waters, the latter showing an array of pores, which could be used for a nanofilter.

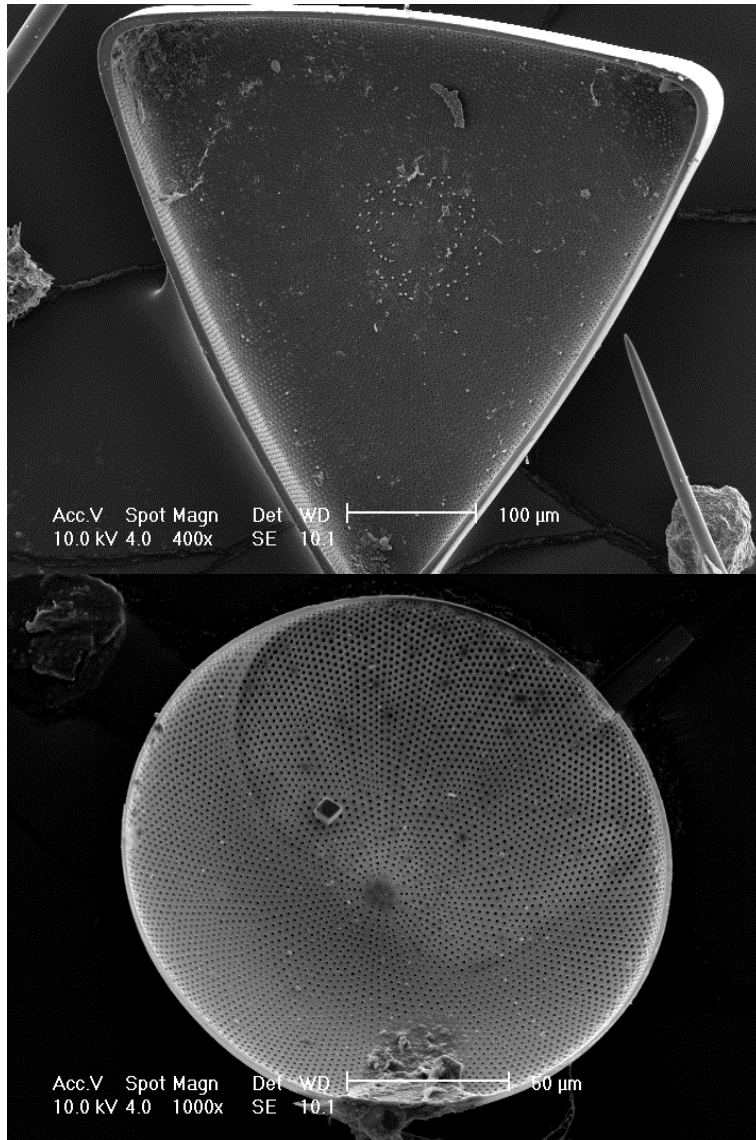
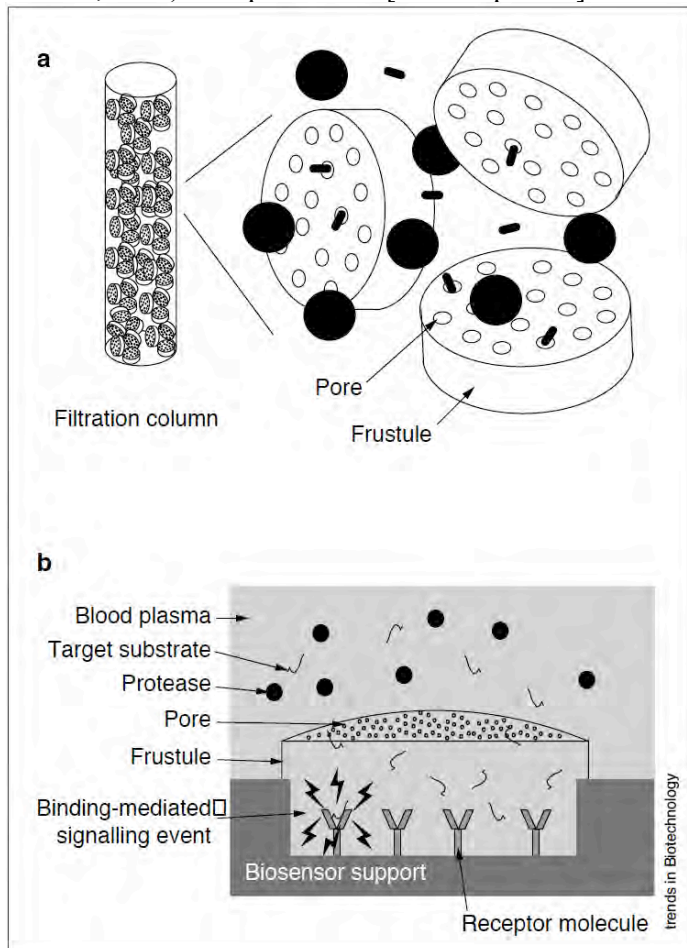


Figure 15. “Filtration applications of diatoms. (a) A filtration column is packed with diatom frustules. Large molecules will pass through the column relatively quickly, while smaller molecules will be able to enter the frustules via their pores and will thus be eluted at a much lower rate. (b) Biosensor filter: in a typical application (e.g. monitoring blood glucose), receptor molecules are contained within a chamber capped by a diatom frustule. Small molecules may enter via the pores in the frustule and bind to the receptors, eliciting a signal. Larger molecules capable of disrupting the signal (e.g. proteases) are prevented from entering the chamber.” From (Parkinson & Gordon, 1999) with permission [to be requested].



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