# DIATOMS FOR NANO-MANUFACTURING

## New Principles for Orientation and Immobilization

By

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Cover:
[Surirella diatom.]

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## ABSTRACT

Diatoms, unicellular micro-algae are one of the most common types of phytoplankton encased in unique cell walls made of silica (hydrated silicon dioxide) called frustules.[*] The cell wall is composed of biogenic silica extruded by the cell which is synthesized intracellular by polymerization of silicic acid monomers. Individual cells could manufacture highly accurate 3D structures in silica from nano meter to micro meter range. Diatoms create scores of very complex three-dimensional structures on a scale which would otherwise be content to create two-dimensional structures. The silica shell could be used as a template to produce a replica in other materials.

In the Vinnova project "Genome based Manufacturing" we investigate how diatoms could be utilized to produce 3D micro-and nano-structures. Different methods to orient and immobilize the diatoms are investigated by us at Swerea IVF. Asymmetrical benthic diatoms such as "*Surirella Sp*" are used for the project. A marine biologist, Anna Godhe at Department of Marine Ecology, University of Gothenburg and a researcher, Anders Blomberg at Department of General and Marine Microbiology, University of Gothenburg are working with the characterization and principles to manipulate the genome of diatoms and to control the structure built by the algae, which is another part of the Vinnova project.

*Microstructure for orientation and immobilization of Diatoms:*

For the orientation and immobilization of the diatoms we designed a PDMS stamp for printing of organic and inorganic materials on glass substrate and a patterned glass plate for light illumination. The diatoms are attracted to light and the working hypothesis is that they will creep towards the light and reside on the illuminated spot.

Motile diatoms move for a while and then slightly adhere to the surface. By understanding the chemotactic, phototactic and adhesion behaviors, we can get the diatoms to move into specified positions, say on a micro-patterned substrate, and then once they are in place convert them into organized arrays of nanotechnology components.

Diatoms have the ability to make their movements directive and useful for them in the slightest way. Photosynthetic in nature, light is the primary energy resource, therefore, orientation and movement can be controlled by light-based mechanisms. Here we use patterns having 20 µm size spots at a distance of 200 µm to observe the motility of diatoms regulated by light source or nutrient. The aim is that the diatoms move to the patterned area, and when they are oriented according to the pattern, we immobilize them. As a result, 3D array of silicon is obtained which could be used for manufacturing of complex micro /nano-structures. In the microelectronics industry fabrication based on conventional 2-D layer-by-layer techniques can be replaced, and low-cost mass production of nanostructured devices with intricate 3-D shapes is possible.

**Key Words:** diatom, bio-mimetic, motility, nanotechnology, silicon.

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*Chapter 1*

# 1. INTRODUCTION

Processes for fabricating three-dimensional (3-D) nanostructured assemblies for use in advanced devices are under progress of development.(1) Precise 3-D fabrication on a fine scale and large scale production of chemically tailored structures is considered necessary for commercialization of sophisticated devices. Such conflicting demands can only be addressed with a revolutionary novel paradigm that could couple the biological self-assembly with synthetic chemistry.

In nature we find a great number of examples of micro-organisms which assemble bio-minerals into intricate 3-D structures. Diatoms (unicellular algae) are the most spectacular among the micro-organisms since each of them assemble silica nanoparticles into a micro-shell with a distinct 3-D shape and pattern of fine (nanoscale) features. Enormous numbers of micro-shells are generated by repeated doubling associated with the biological reproduction (for example: > 1 trillion 3-D replicas can be reproduced in just 40 cycles approximately). Such accurate precision and massive parallelism make it attractive for manufacturing of complex 3-D structures which is not possible by using current fabrication techniques.

## 1.1. MANUFACTURING OF NANO-STRUCTURES

In manufacturing, control of the nano-structures, could be achieved in two ways, either a top down manufacturing or bottom up by self-assembly. The control of the structure in bottom up manufacturing is limited to the chemical interaction between molecules. Near-ordered structures can be obtained and these near ordered structures can be repeated over larger distances but complex 3-D structures cannot be manufactured.

Micro-fabrication process capabilities have to be expanded where various geometric forms and different materials are covered, which can serve the requirements of emerging multi-material products. Process for manufacturing components and devices which have complex 3D features from a single material in the micro/nano length scale are required for higher throughputs. The developed process should be highly flexible and also provide capabilities for large scale manufacturing simultaneously, where cost is also optimized in the fabrication lines.

Nature has solved the problem of how to control the macro-structure manufacturing in the bottom-up method!

Inspired by nature, naturally occurring systems and processes can be mimicked by bio-mimetic engineering, which creates novel advanced structures, devices and materials where molecular self-assembly acts as a key link between physics, chemistry and biology. Forthcoming conventional technologies and insufficient performances are driving science to seek new engineering solutions based on nature's biological concepts and methods.

The combination of biotechnology with manufacturing techniques (figure 1.1) at molecular levels – can result in nano-biotechnology, where manufacturing at a micro/nano scale is very precise. In this aspect Diatoms are very remarkable model systems in order to study how nature manufactures complex 3D Micro-Nano structures, because Diatoms manufacture shells of silicone dioxide in very large variations. Bio-inspired materials design and manufacturing can be realized in different ways. Thus, high-value-added ceramic, metal or polymer products can be generated by utilizing the controlled shapes and fine features of the diatom structures.(2)

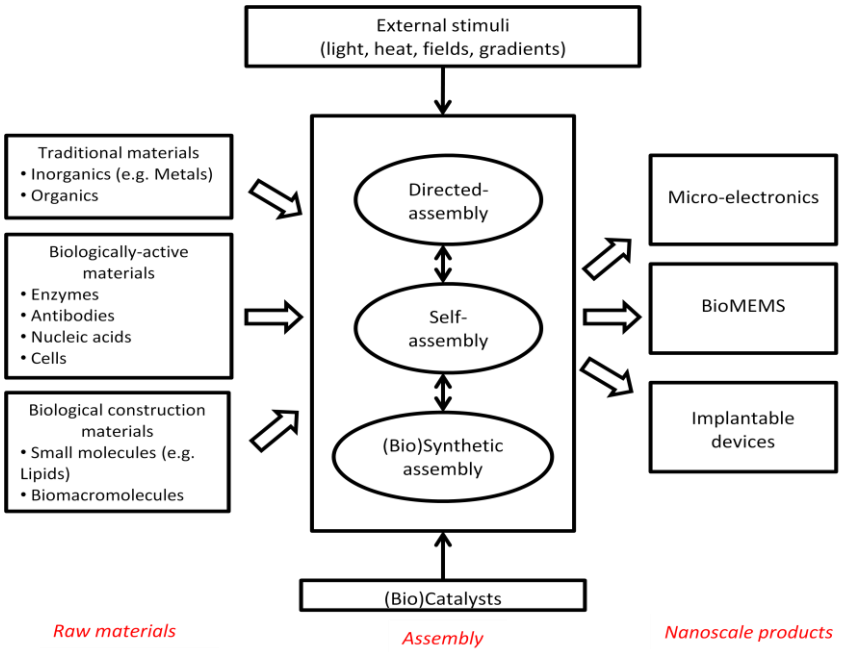


**Figure 1.1: Biological construction materials combined with micro fabrication**[3]

Bio-inspired manufacturing is an emerging technology which gives revolutionary advances in the fabrication of micro- or nanostructured materials and devices. A future direction in nano-manufacturing is based on the interface between bionanostructures and bioinspired structures resulting in, multifunctional and adaptive nanostructures. This is a joint exploration of nanotechnology and biotechnology, as genome controlled diatoms could also be grown intended for nano-manufacturing in silicon retaining their complex structures as it is. For example, Zhong Lin Wang shows silica based nano-wires can be grown using solid-vapor phase process (Figure 1.2a), but in nature such structures occur naturally, like silica based diatoms, these genetic engineered structures have precise reproducibility and also produced in large numbers (Figure 1.2b).[4] This shows that the best example of nanomanufacturing is found in nature.

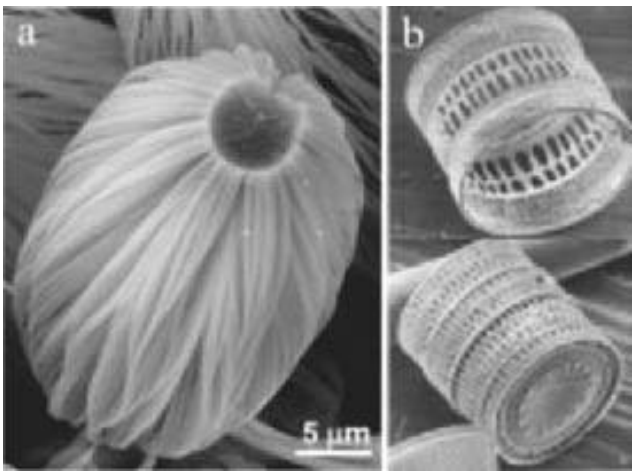


**Figure 1.2: (a) silica nano-wires synthesized by solid-vapor process (b) silica-based diatom structures formed in nature through genetic engineering** [4]

Nano-manufacturing supports tailor-made products with critical nanometer-scale dimensions which are produced using massively parallel systems or self-replication. For the technological advances, industrial nano-manufacturing poses a serious challenge because of the limitations of the techniques like optical lithography and manipulation techniques. Therefore, to meet the future manufacturing needs, possible solutions could be designed and controlled self-replication (genomic control), precise and simultaneous fabrication of large number of devices under controlled conditions with repeatability and at low cost and flexibility.[4]

### 1.2. AIM OF THE PROJECT

For the orientation and immobilization of the diatoms into specific positions, a top down approach was developed. A soft imprint tool for manufacturing of pattern and an array of holes in a mask has been designed and fabricated; the fabrication was done by *Technological Center Tekniker*, Spain within the EUMINAfab project.[5]

The aim of the thesis project is to achieve the positioning of the diatoms as per the designed patterns. For achieving good results and testing different alternatives, two designs are made, one on titanium-on-glass masks with one hole or two holes adjacent to each other on which light can be illuminated to observe the diatoms move towards the light into the positions as per the pattern and another pattern is such, which is helpful for printing of silica gel or any other organic or inorganic material.

The project goals intended to be achieved are based on the following considerations:

- Extensive literature survey on Diatoms and recent developments in the use of Diatoms in micro and nano technology for nano-manufacturing.
- Finding suitable approaches for the orientation and immobilization of the diatoms, to have better control of them.
- Learn the fabrication and handling of PDMS stamps for printing of organic and inorganic materials on glass substrate and a patterned glass plate for light illumination and observing the reaction of the diatoms.
- Evaluation of the methods used, depending upon the results obtained.

Inferior reproducibility and controllability of man-made nanomaterials can be overcome in the genetic-engineered natural materials, having precise reproducibility and large amount production. Hence, we are testing nature's secret of manufacturing nano and micro structures which could serve the demands of complex 3D nano-manufacturing, with integration of engineering, science and biology.

### 1.3. METHOD

As described above, the purpose of the thesis work has been to examine various aspects to orient the diatoms into required positions, so the obatined array could be of use in the nano-manufacturing. An extensive literature survey on diatoms in the field of nanotechnology, and the biology of diatoms, is done in order to find suitable approaches for the orientation and immobilization of the diatoms. The process could be summarized as follows:

i. Fabrication of Silicone masters and Titanium-on-glass masks.

ii. Incorporating nutrients into Sylgard 184 elastomer.

iii. Analyzing the release of nutrients from the elastomer over time.

iv. Designing a process for transfer printing the material on to a glass surface.

v. Analyzing the growth of diatoms on different modified surfaces.

vi. Conducting the experiments.

vii. Interpreting the results to evaluate if the desired pattern is obtained.

It has been possible to gain knowledge of the process followed for the fabrication of the Silicone masters and Titanium-on-glass masks done at *Tekniker, Spain* and get trained to make own PDMS stamps and handling of the masters, as part or the EUMINAFAB project. A literature survery has been carried out in different areas specifically, such as to understand the reasons responsible for the movement of diatoms, for incorporating the nutrients into an elastomer and also growth of diatoms on modified surfaces.

*Chapter 2*

# 2. DIATOMS

## 2.1. INTRODUCTION

Diatoms, unicellular micro-algae, are microscopic photosynthetic plankton found almost all over where there is water. These are single-celled autotrophic organism having a highly ornate siliceous wall. They can occur in large amounts and forms (as in Figure 2.1), and the number of species is estimated to be over 100 000. Each type of diatom has a different formation and its own appearance as it can be seen in the figure below. No two species will have the same properties and features which is an attractive feature for the changing generations in the technological world.

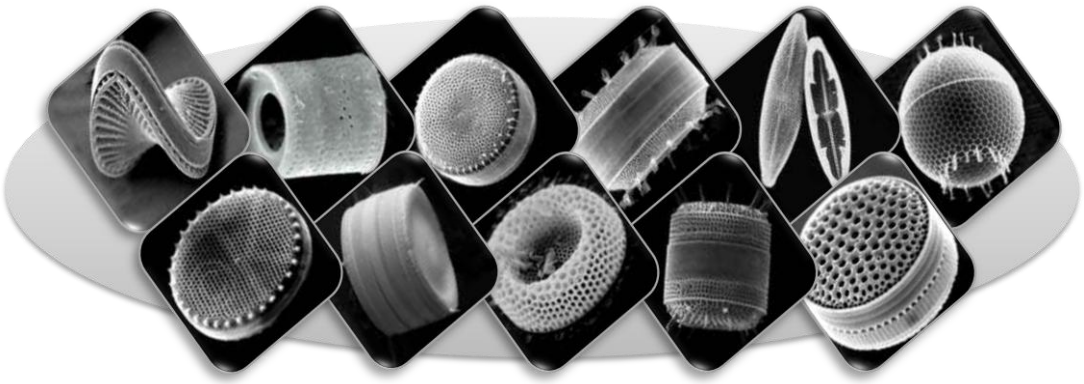

**Figure 2.1: Diatoms**

It can be roughly calculated that there are around 6 million diatoms per cubic foot of seawater. Organically it is very important because their photosynthesis accounts for approximately 25-30% of the organic carbon sequestration and thus a significant part of the world's oxygen production.(6) Thus diatoms take in Carbon dioxide form the environment and produce Oxygen.

### *Diatoms Ecology*

A major constituent of the plankton family, diatoms are free floating micro-organisms in marine or fresh-water environments. These are usually freely floating, but not all; some may also cling to surfaces like aquatic plants or the body of turtles or whales. Sometimes they also grow in moss outside the buildings that have been left untouched for a long time. Diatoms of any kind may occur as individual cells or form chains (colonial chains) as in figure 2.2 below.

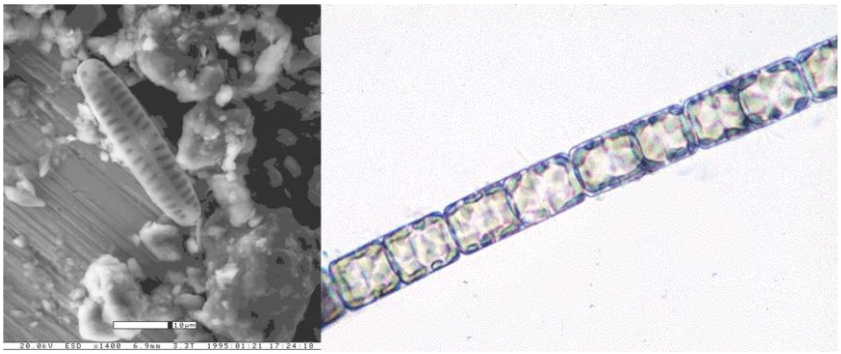

**Figure 2.2: Diatoms occur individually (left) or form long colonial chains (right)** (7)

To survive, diatoms require only light, heat and some basic nutrients. Diatoms belong to, as a part of the ecological assembly known as phytoplankton. These require dissolved inorganic nutrients, including $CO_2$, nitrogen, phosphorous, silicon and a wide range of metals and vitamins, to photosynthesize and reproduce.

### 2.1.1. STURCTURE OF THE DIATOM

The cell is encased in unique cell walls known as frustules. This frustule or the cell wall of the diatom is composed of biogenic silica, extruded by the cell which is synthesized intracellular by polymerization of silicic acid monomers. Individual cells could manufacture highly accurate 3D structures in silica from nano meter to micro meter range. Diatoms already create scores of very complex three-dimensional structures on a scale which would otherwise be content to create two-dimensional structures. Thus the shell is basically composed of SiO2 and can create fanciful forms.

As silica in either form is used by a number of organisms to form their exterior coverings, diatoms, make their vegetative cell wall (frustules) using strongly differentiated plates (valves) and bands connecting the plates, usually the main component being silica (Figure 2.3).[8] A valve and a set of associated bands are composed in each half of the frustules. Both the valves and the bands are highly structured, but typically, valves are more ornamented and structured than the bands. The top half valve is known as the epivalve and the bottom is the hypovalve, slightly smaller than the epivalve. As a result, the entire frustule is built up of silica (hydrated silicon dioxide) and this is produced by the diatom itself under its natural conditions, which shows that there is no pre-requisite care to be taken for the growth of these organisms.

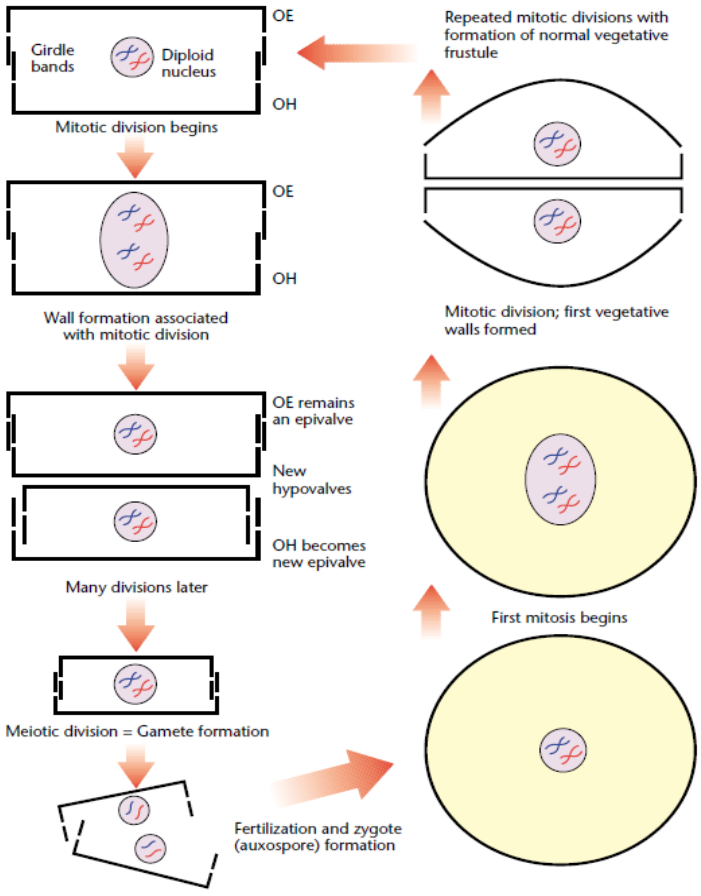


**Figure 2.3: Diatom life cycle (OE: original ephivalve, OH: original hypovalve)** [8]

### 2.1.2. REPRODUCTION PROCESS

Vegetative reproduction (Figure2.4) (asexual) is the main mode of reproduction, which results from typical mitotic division of the diploid nucleus. Each of the new daughter cells formed after reproduction keeps one half of the frustules (a valve and girdle band set) from the parent cell and forms the other new half by itself. After the cell division, the new cell formed is usually smaller than the original cell; vegetative division usually gives cells of various sizes. Therefore, it is very common to find cells of different sizes in a single culture after it is left for a few days. Some of the species may reproduce at such high speeds that, one individual gives rise to 100 million descendents in a month probably.[9]

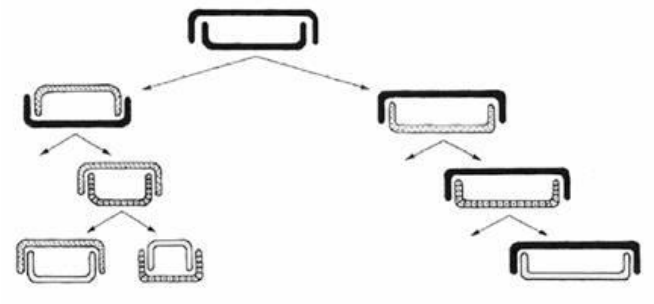

**Figure 2.4: Vegetative reproduction (at each cell division the new cell is formed inside the parental cell causing the size to decrease)**[10]

The single celled organisms, diatoms, have intricate skeletons which can be either elongated with bilateral plane of symmetry or round with radial symmetry. Major Groups: traditionally classified into 2 groups, depending upon the valve symmetry: (i) the centric diatoms or centrales round in the outline, prefer floating as the phytoplankton (ii) the pennate diatoms or pennales, long and narrow are accustomed to living on the bottom or other substrates in the ocean. Some of the diatoms are asymmetrical, but will belong to any of the two categories depending upon their skeleton structure.

Thus diatoms are a fantastic role model for building micro and nano systems. Diatoms are small factories that build nanostructures with very high accuracy, and can nurture 3D nano-and microstructures of 200-50 microns with features down to 10nm in the same unit. Also some of these diatoms form colonies and orient themselves in relation to one another and creating much larger structures.

Ranging in size between 2-2000 μm, these diatoms create their own silica walls at ambient temperatures and pressures. Cells walls enclose the protoplasm, forming a hat-box-type structure as shown above. Nanometer scale features in the diatoms are under strict genomic control, which are difficult to reproduce using current manufacturing techniques.

### *2.1.3. SURIRELLA Sp*

In this project we use diatoms to produce micro- and nano- structured systems and investigate the principles of how to use the genome and proteins to guide and control the manufacture of 3D micro and nano structures. Among the 100 000 species, we use the unsymmetrical pennate diatom "*Surirella Sp*" (Figure 2.5), a benthic diatom approximately 45 μm long mostly found in fresh waters. There is a raphe or a groove between the two halves (top and the bottom in the box like structure) usually lined with cilia down one side to help glide along the surface.

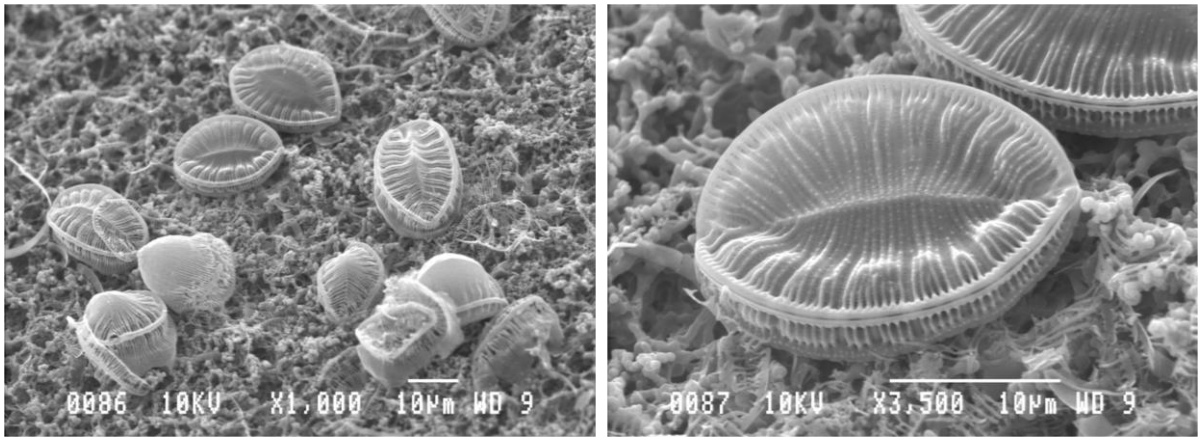


**Figure 2.5: SEM pictures of *Surirella* used in the project**

A protective layer covers the skeleton of a diatom (or frustules) [skeleton structure as in Figure 2.5], the coating contains organic matter (proteins and carbohydrates), which does not dissolve easily in water, but can be removed with no difficulty (by a simple recipe followed to dissolve the coating layer; as explained in the experimental section for SEM analyses of *Surirella*) to expose the bio-silica. When the protective layer is dissolved, the frustule structure is as in figure 2.6.

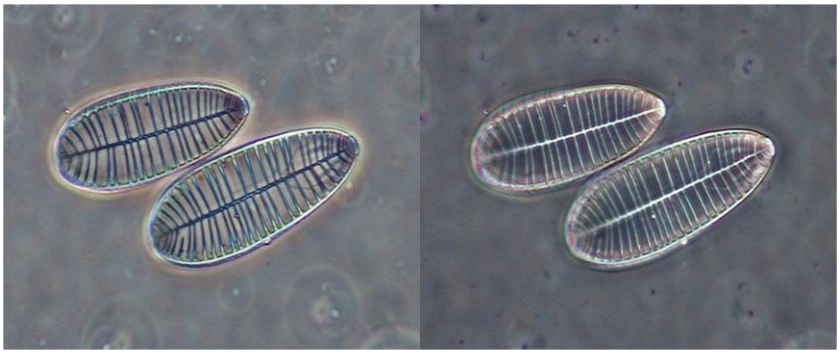

**Figure 2.6: *Surirella* diatom frustule assembly viewed in a microscope under positive and negative phase contrast illumination; Positive (left) Negative (right)** [11]

## 2.2. DIATOM LOCOMOTION

The cell is enclosed in a beautiful sculptured siliceous wall, where 2 halves or valves are interconnected by girdle bands (Figure 2.7). They can be described in two ways, (i) girdle view where the girdle bands are seen and (ii) valve view where the face of the valve is observed. Hopkins using Froude's Law (square of maximum velocity is proportional to length) showed that "diatom velocity is not related to its length".[12]

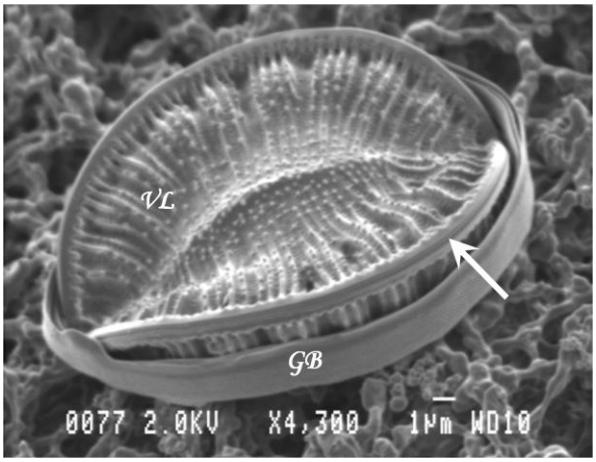


**Figure 2.7: Scanning electron micrograph of *Surirella* in valve view, showing the ornamented face of the valve (VL), the girdle bands (GB) and the raphe (arrow)**

More or less all of the motile diatoms are attached to the surface, where the raphe is present so that movement can occur. Moreover only one raphe system per a motile diatom is required for locomotion. Motile diatoms that are attached to a substrate have a few advantages, such as (i) be in position in moving water, (ii) avoid being buried by moving upwards in sediments, (iii) moving to colonize vacant areas and (iv) moving to areas with more light and nutrients.[12]

In pennate diatoms, movement is mostly based on the structure and the shape of the raphe. A number of theories exist in relation to, how locomotion is produced, but all conclude at single phenomenon that, the raphe system connected to the siliceous surface of the diatom is primarily responsible. The raphe present between the two girdles overlaps small bundles of microfilaments which generate the gliding motion in the *Surirella* and the raphe is responsible for propulsion.[13]

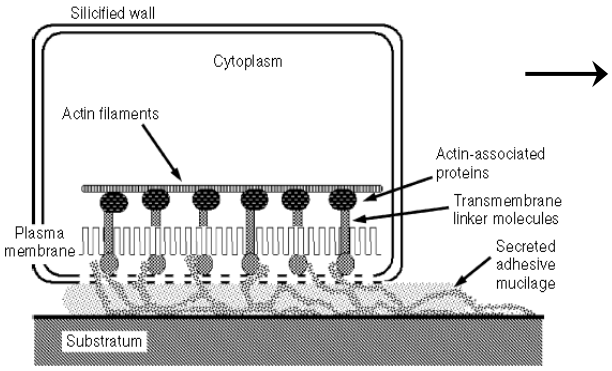


**Figure 2.8: Schematic shows cell propulsion in relation to adhesive mucilage** [14]

When the cell is propelled forward adhesive mucilage is secreted from the raphe at the point of contact with the substratum, (Figure 2.8) and laid down as path in the forward direction thus helping in gliding process. Before the motility begins a contact is established between the raphe and the substratum (as in Figure 2.9) and then this seal acts as guide for the direction of the diatom movement and also as a support which helps gliding (cell motility).

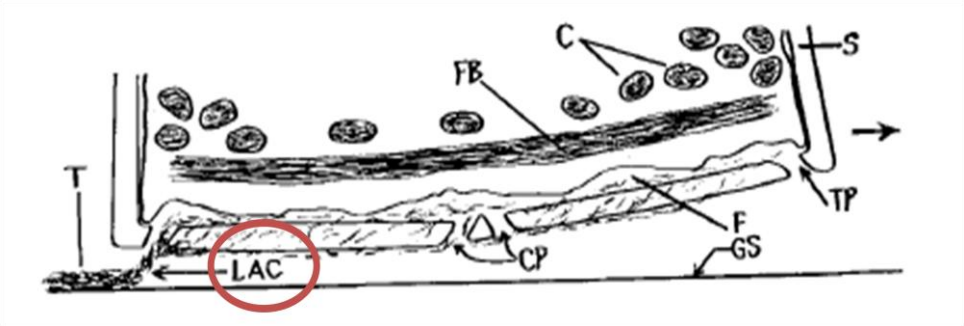


**Figure 2.9: Raphe plane section of a diatom moving upon a plane glass surface (GS) showing point of locomotor-adhesion contact (LAC)** [15]

To explain diatom locomotion, the functioning of the raphe system is briefly described. It needs to receive an indication for stimulating the fibrils responsible for locomotor to start its work. The raphe system of the diatom reacts to photo-tactic or photophobic changes, or nutrient availability; or needs a mechanical push like a tidal fluctuation, and then the system is forced to initiate movement. Gliding speeds range from 0.1-25 µm/sec in rapid diatoms, vary depending upon the substratum type and environmental factors.[14][15] Speed of *Surirella biseriata* ranges between 6-10 µm/sec.[16]

## 2.3. ENVIRONMENTAL FACTORS INFLUENCING DIATOM MOTILITY

"Diatoms are observed to frequently change direction and reverse itself along its path".[17] Movement responses can be seen in the diatoms when they respond to external stimuli such as light, temperature, nutrient availability, salinity and more. From the cross-section in figure 2.10 we have the adhesive strand from the raphe and the mucilage layer on the girdle bands responsible for movement by the diatom. Responsive to the environmental signals, relative rapid movement changes are discussed below, but it differs from species to species and here we only see a universal depiction.

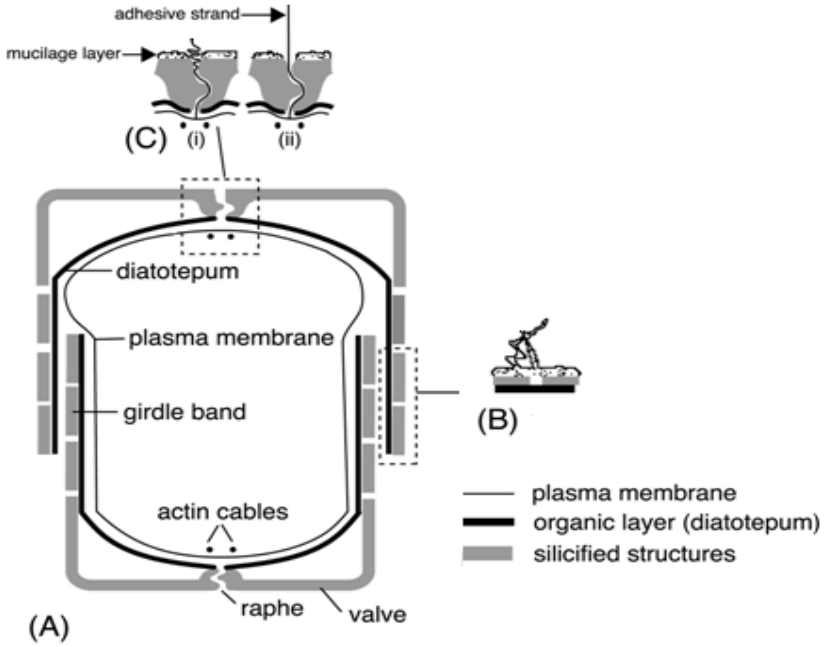


**Figure 2.10: (A) Cross-Sectional diagram of frustule (B) Mucilage layer on the girdle (C) Adhesive strand secreted from the raphe anchored to the plasma membrane** [18]

### *A. Light (Photo-stimulated effects)*

Light plays an essential role for rapid changes in the cell direction. The reason could be the existence of a photo-detection system at the tip of the cell. Different species will have different thresholds that will trigger migration; some are dominant at high light levels or during early mornings or midday. But in any case they only move when they need light for photosynthesis or will stay away to avoid damaging effect of high irradiance.[19] Light irradiation can also be used to shift the distribution of diatoms in a particular area. Some kinds of diatoms can also be found to be accumulating in area of low to moderate light and avoiding high intensities.[17]

### *B. Nutrients*

Nutrients are one of the external stimuli that can bring movement responses in the diatoms. Availability of nutrients in the micro-layers of the sediments might be directly proportional to the species vertical spatial distribution. Also dominance of cells could be observed generally in the anoxic layers of the water bodies usually, because this layer is rich of inorganic forms of nitrogen ($NH_4^+$ or $NO_3^-$). It may also be that some species could migrate along a particular nutrient whereas in its absence move randomly. Furthermore, cells in different phases of cell division may also be found in large numbers in the nutrient rich layers, which is suitable for growth and division.[19] Additionally, inorganic compound like calcium may have a significant impact on the motility of the diatoms, if it is not internally regulated and is in high or low dose – may inhibit the motility.[20]

### *C. Temperature Effects*

Drum and Hopkins tested *Pleurosigma angulatum* (a type of diatom) samples to investigate the motility and adhesion of diatoms on a substrate at different temperatures. Living cells, adhered to the glass surfaces when the temperatures were changed from 20°C to 55°C, when heated to 40°C they also remained attached, if not disturbed. Diatoms even exhibited adherence even when the sample was cooled down to -1°C, where the medium freezes. The heated diatoms, when cooled back to the room temperatures exhibited normal motility as usual. But in all cases, if the cells were dead during these fluctuations, they did not re-adhere to the glass if detached.[21] Rapid rise in temperatures or tidal changes could have an impact on the diatoms.

### *D. Salinity variations*

The gliding movement of the diatoms is mainly affected by the salinity levels. For a short-time in the hypo-saline condition alteration is caused in gliding whereas in extreme hyper-saline condition cessation of motility happens and is very fast, almost within 5 sec. Therefore hypo- or hyper- saline environments, gliding speed is decreased than in the standard media.[19]

### *E. Surfactants*

Addition of surfactants like detergents or soaps will put an impact on the locomotion of the diatoms but streaming and adhesion are not affected. Lowering surface tensions do not prevent adhesion but motility is reduced and the cells are alive in state, if the same cells are gently washed and given fresh media then the motility is returned in about 2-3 minutes. So, to slow down the fast moving diatoms, surfactants can be used in case it is required for specific purposes. For example, to immediately stop the movement of the cells, a surfactant with dilution of 0.1 to 0.001% might be required, and at 0.0001% dilution the cells might start giving jerks and may show some illness which makes them dull and if the dilution is 0.00001% then motility is usually normal, since it is a very low level of detergent in this case.

### *F. External Layers*

Locomotion is withdrawn in case there is any external layer such as the locomotor secretion material covering the raphe system of the *Surirella*, because there is no likelihood for raphe-substratum contact.

### *G. Drugs*

For diatom locomotion secretion of mucilaginous material or extracellular polymeric substances (EPS) is essential. Locomotion is inhibited by the addition of drugs because it stops the flow of the secretion material. Marine fresh water diatoms slow down and stop after 30-60 sec (approx.) if some kinds of drugs like Isoprenaline (0.1%) or Ephedrine (0.1%) are used. But the movement is gained back in some cases after a wash just in time or they are irreversibly affected. Strong dosage of drugs for long times could either kill the diatoms or they may remain alive without movement. Few very sensitive drugs (like additive drugs used for preservation or the ones used in medicines) may have no effect on motility and adhesion of the diatom.[21]

*Chapter 3*

## 3. PROSPECTS OF DIATOM NANOTECHNOLOGY RESEARCH

Diatoms have a very interesting model system with high potential in the nano-structured production as there are almost 100 000 different species with unique frustule morphologies. Figure 3.1 shows the large variation in the frustule structures. With multifunctional properties, $10^5$ different diatom species are formed with hierarchical architectures, ranging from nanometers to micrometers. The frustule structure could be used in building advanced devices such as light harvesting, photonics, molecular separation, sensing, and drug delivery systems. The frustules have, because of the architectures a lot of optical mechanical and transport properties that are useful in making these highly developed devices.

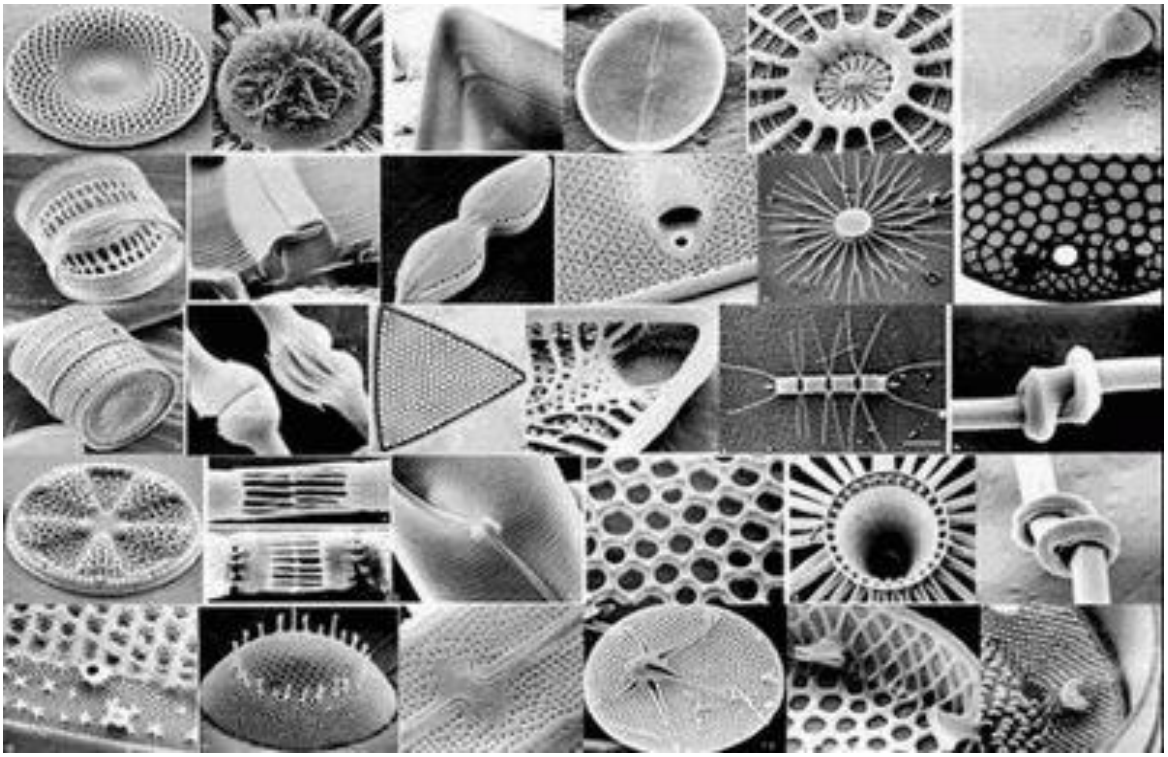

**Figure 3.1: Diversity in shapes and structures of diatoms** [22]

But the question is whether there are any other principles, such as: chemical / thermodynamic / kinetic influences which govern the diatoms, and how they are grouped into macro-structures such as forming clusters and colonies. It is especially interesting to focus on things such as; diatom frustules' potential for advanced materials with respect to their intricate structure, synthesis of novel silica-based material by biomimetics, and also principles responsible for colonizing; and how these can be exploited to serve as alternatives in nanofabrication techniques, sensing devices, etc. [23] In this project we will particularly study the diatoms' natural and induced ability to orient itself and their possibility to attach to the surfaces.

### *Diatomaceous lessons in nanotechnology and advanced materials*

For the last few decades, use of silicon and silica has been intense, and the diversity existing for innovation with this element raises scope for research. In 1988, Richard Gordon invented the word "Diatom Nanotechnology", referring to the ways diatoms create 3D nanostructures by controlled deposition of silica in their skeletons. Minute structures made by the diatoms which are beyond the

capabilities of materials scientist are attracting the attention of nanotechnologists to learn a large number of concepts from them.[24] Current research is focused on fabrication and self-assembly methods for shaping elemental silicon in nanometer to micromenter length ranges, for widespread use in electrical, optical and structural materials. Various fields and application domains for diatoms in biotechnology and material sciences are creating interest for the nanotechnologists (Figure 3.2).

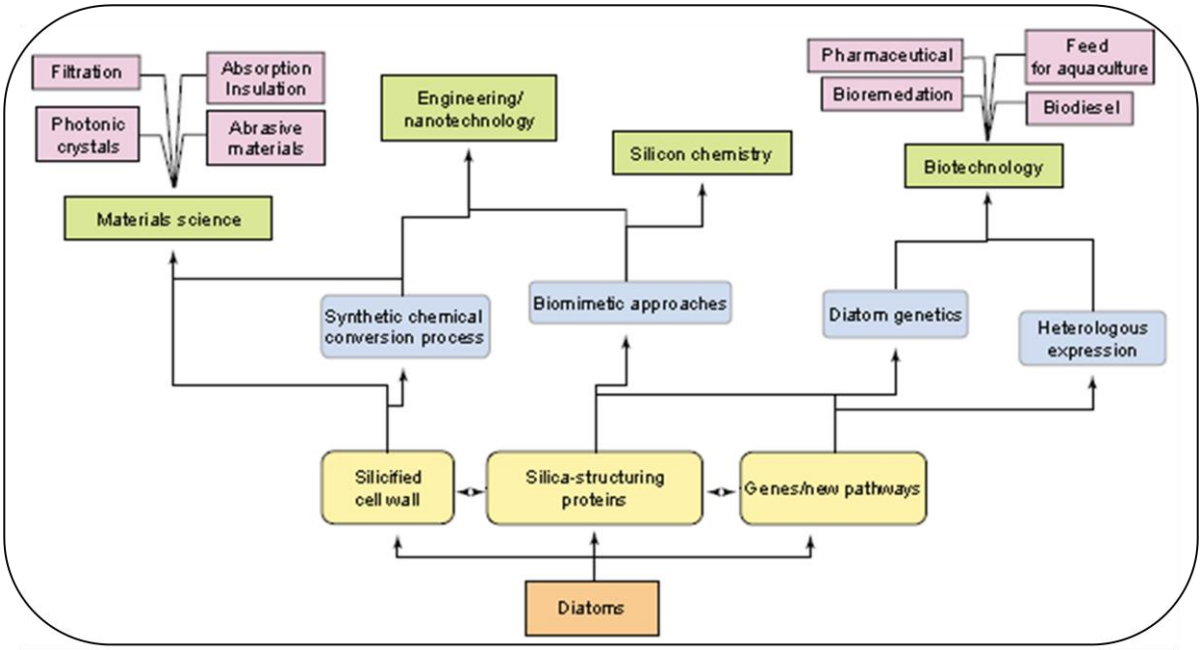


**Figure 3.2: Various fields and applications for diatoms research** [25]

Joanna Aizenberg of Lucent Technologies' Bell Laboratories in Murray Hill, N.J., says, "We can think of diatoms as living silicon chips". Semiconductor manufactures build micro- and nanoscale features for use in electronics and optics, a costly and time-consuming undertaking. Comparatively, diatoms build structures out of silicon with more proficiently. Although diatoms are not likely to put the semiconductor industry out of business in the near future, their capability to create new materials with complex structures on a miniature scale may possibly serve as the foundation of a powerful technology in the near future. Because, by regular means, making of silicon chips or other electronics require a lot of harsh chemicals and also produce much waste, whereas diatoms produce the same kind of results without using any chemicals and at ambient temperatures.[26]

## 3.1. DIATOM BIOMINERIALIZATION – FORMATION OF BIOLOGICAL NANOSTRUCTURES

Diatoms generate their cell walls by silica biomineralization, composed of silica and organic molecules. The cell wall has a complex structure. Inorganic components constitute about 97% of the cell wall compounds, particularly silica along with trace quantities of aluminium and iron. And the main organic components constituting the diatom biosilica are silaffins, proteins posttranslationally modified by long-chain polyamines and oligo-N-methyl-propyleneamines. To achieve the biomineralization particular interactions , choosy organic moieties and biocompatible minerals, are to be evolved.

The frustule of the diatom gets its flexiblity and toughness from the materials it is composed of; silaffins and polyamine proteins co-precipitated with slicic acid available in the aqueous environment form the organic material of the diatom frustule. This is stronger when compared to

the comerically available bulk silica, one of the most brittle materials known. Along with the nanoporous structure it also enhancess the toughness in addition to the materials forming the frustule.[27] [28]

Silica becomes increasingly used in chemical, pharmaceutical and nano-technological processes, which causes an increased demand for well-defined silicas and silica-based materials. Production of highly ordered silica under economic satisfaction, with cheap starting materials and ambient conditions is the current research target from the (nano-to-microscale) manufacturing perspective. This is already realized from the formation of diatom biosilica, where highly hierarchical ordered (Figure 3.3) meso- and macro- featured silica structures are produced.[29][30]

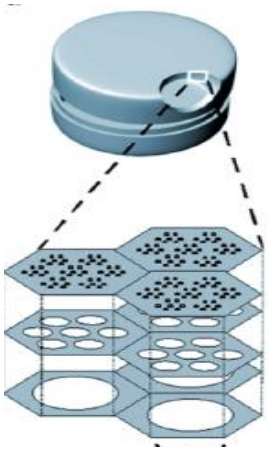

**Figure 3.3: Hierarchical structure in a diatom along the hexagonal grid** [30]

Rigid cell walls (frustules) out of amorphous silica are formed by diatoms, which is massively parallel and precise 3-D hierarchical assembly.The diatom silica structure formation is dynamic and nonequilibrium process, where optimization is necessary not only in the fomation stages but also in the final phase. Produced by natural biomineralization using organic templates with controlled growth of the inorganic phase; with well-defined structures, arranged from the nanometer to macroscopic length scale, we have diatoms, shells, bones and teeth as unique examples. Ability to fabricate inorganic materials into complex hierarchical patterns by bottom-up self-assembly process is possessed by diatoms. Inspite of our limited understanding of the hierarchical complexity found in nature, strategies for mimicking nature have partially succeeded in synthesizing human designed bio-inorganic composite materials.[31] [32]

A major aspiration for this research is to utilize the diatom expertise in biogenic silica construction to build up strategies for bio-inspired nanofabrication of silicon based equipment. In this project, we try to report a synthesis methodology for utilising the highly ordered slica structures formed by the diatoms, and arranging them into a definite pattern, which could be useful for many mechanism such as slow dispensing of medicines or other nanotechnological applications. It can be, if possible, further generalized as a rational preparation scheme with well-defined multiscale architectures for applications into biotechnology as well as nanotechnology.

## 3.2. CONVERSION OF DIATOMS INTO 3-D STRUCTURES WITH NEW CHEMISTRIES – BIOMIMETICS

Diatoms have developed elegant solutions for precise 3D manufacturing, enabling the low-cost mass production of micro/nano-devices with complex structures under physiologically compatible and environmentally gentle conditions. Replacing the existing nanofabrication techniques, such as 2D

planar lithography, they are beneficial because of minimal energy usage and minimal waste production. Understanding about the bioprocesses available for the synthesis of nanomaterials may perhaps give the potential to design and develop new nano-sized devices.

Biomimetics is the extraction of good designs from the nature, where diatom biomimetics stands high in the materials community. A number of strategies for creating materials with outstanding functional properties are evolved from nature. There have also been many experimental works where the diatom nanostructures are built or copied to create complex architectures in 3-dimensional, which also do not fail due to friction, wear, adhesion or lubricant loss such as man-made micro-electromechanical systems (MEMS).

### 3.2.1. BIOCLASTIC AND SHAPE-PRESERVING INORGANIC CONVERSION (BaSIC)

As the frustule of the diatoms with hierarchial 3D porous structures is well defined and could be best appropriate for many applications, for instance into heterogeneous catalysis or seperation technologies or more. These kind of features have inspired the design and production of new nanomaterials by the maintenance of diatom nanostructure and modification of the material chemistry. Thus, there are number of processes (as in Figure 3.4) which could be used to produce composite materials having highly defined hierarchical structures from a large variety of easily and commercially available diatom species.

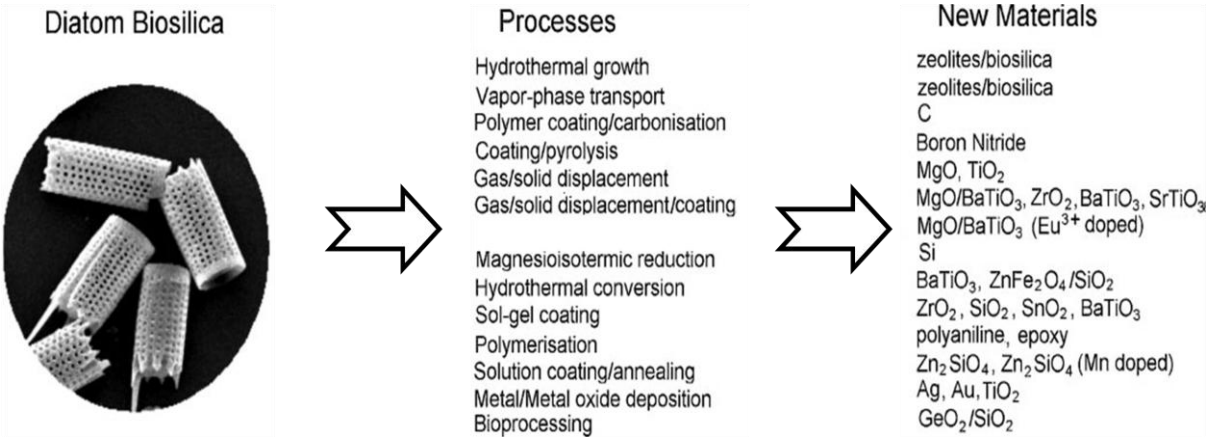


**Figure 3.4: Summary of process, for producing 3D nanostructured materials** [23]

A strategy known as BaSIC (bio-clastic and shape preserving inorganic conversion) has been devised by *Sandhage et al*. where gas/silica displacement reactions, conformal coating, or a combination of all are used for converting the diatom frustules' chemical composition without losing the bio-assembled 3D morphologies.

- ***Gas/Silica Displacement Reaction***

Silica in the diatom frustule is replaced by either oxidation/reduction or metathetic displacement reactions. Conversion of $SiO_2$ – based *Aulacoseira* diatom frustule (figure 3.6(a)) into MgO or $TiO_2$ replicas was based on gas/silica displacement reactions. For the conversion, diatom frustules along with solid Mg/solid $TiF_4$ granules are sealed inside metal ampoules at opposite ends as seen in figure 3.5. Then the sealed ampoules are heated to 900°C/350°C and held for 1.5-4hrs/2hrs. Finally tubes are cut open, reacted frustules (figure 3.6(b,c)) are removed and ready.[33] Reaction (1) shows the oxidation-reduction displacement reaction used at 900°C to convert $SiO_2$ frustules into MgO-based

replicas and similarly reaction (2) shows the metathetic displacement reaction used at 350°C to convert into $TiO_2$ replicas.

$$2Mg(g) + SiO_2(s) \Rightarrow 2MgO(s) + Si(s) \quad (1)$$

$$TiF_4(g) + SiO_2 \Rightarrow TiO_2(s) + SiF_4(g) \quad (2)$$

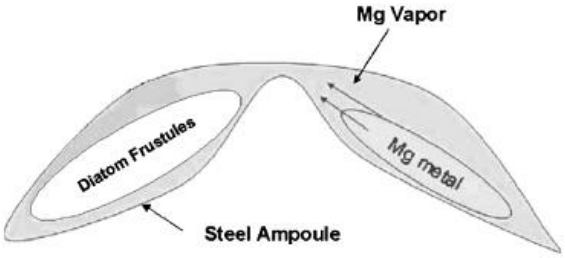


**Figure 3.5: Schematic of the steel ampoule configuration used to seal diatom frustules and Mg/Ti metal** [33]

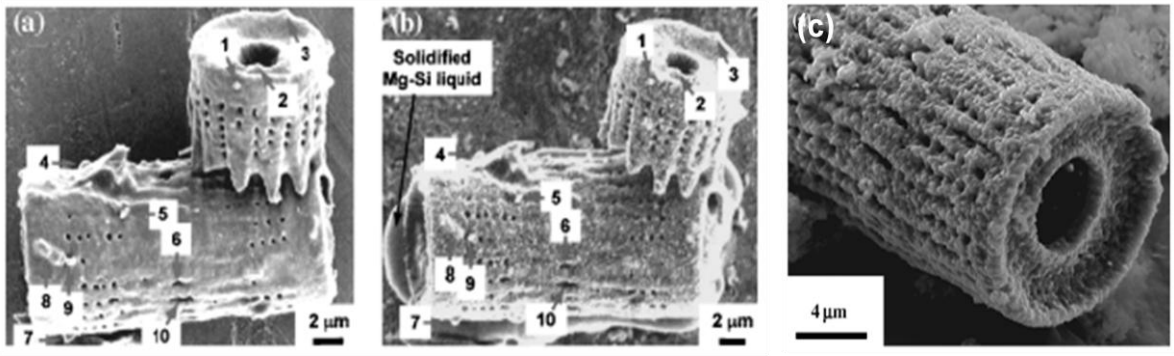


**Figure 3.6: Secondary electron images of (a) $SiO_2$-based *Aulacoseira* diatom frustules (b) MgO converted replica (c) $TiO_2$ converted replica**[33]

- ***Conformal Coating Methods***

Coatings of epoxy or zirconia are applied to the diatom frustules by using wet chemicals. The procedure for both types is; firstly frustules are immersed in dilute precursor solution dissolved in volatile solvent, then upon removal of the frustules from the solution a solvent is allowed to evaporate forming a thin conformal coating. For polymer coatings, it is allowed to rigidify by cross-linking and for zirconia coatings by calcinations. Finally underlying SiO2 is dissolved, just leaving coating-based replicas.

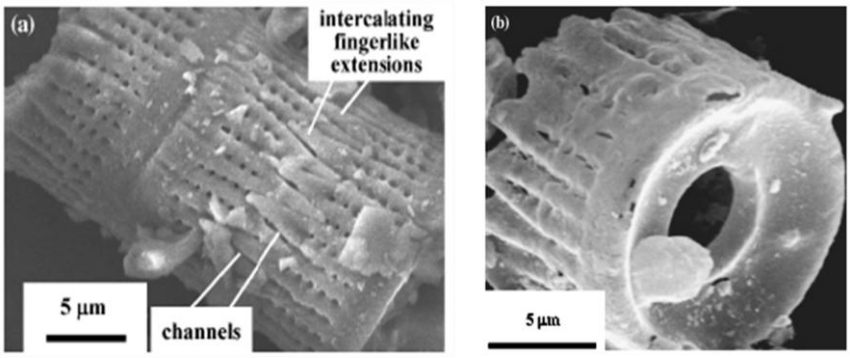


**Figure 3.7: (a) Epoxy replica (b) Zirconia replica of *Aulacoseira* diatom frustule**[33]

In case of polymeric replicas, epoxy mixture is dissolved in acetone, in which *Aulacoseira* diatom frustules are stirred and removed after 15 min, allowing acetone to evaporate. They are cured at room temperature and then exposed to HF solution to dissolve SiO2. The result is epoxy replicas (figure 3.7(a)). For ZrO2 replicas, frustules are exposed to ammonium hydroxide solution, then immersed in ethanol solution containing zirconium n-propoxide and left for 6hrs and taken out for heating in air, up to 650°C. Then immersed in Sodium hydroxide solution to dissolve SiO2, giving zirconia replicas (figure 3.7(b)). By these means the shape and fine features are very much preserved as it is.[33]

- ***Combined Use Of Displacement Reaction and Coating Methods***

To synthesize composite frustule replicas, displacement reactions and conformal coating methods are combined. $BaTiO_3$ coated MgO replicas are produced. By initially using the displacement reaction (1) MgO replicas are produced, and then immersed in ethyl-based solution containing barium and titanium. Left for 5hrs and then heated at 700°C for 1.5 hr. these replicas will avoid the chemical reactions that may occur due to incompatibility, where MgO converted replicas are chemically inert onto which sol-gel coating may be generated. In the $BatTiO_3$/MgO (figure 3.8) composite frustule, the overall morphology of the initial diatom frustule is conserved.[33]

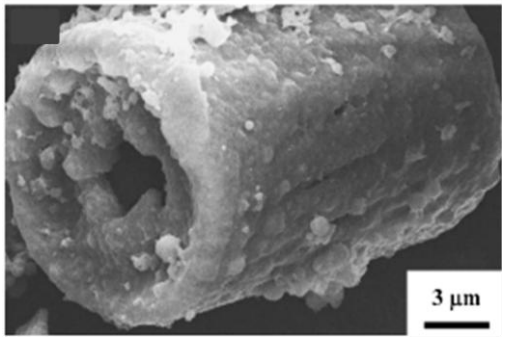


**Figure 3.8: Composite frustule replica; BaTiO3-coated MgO frustule** [33]

By using the approaches, nano-engineering efficiencies of living cells such as diatoms are harnessed which can serve as alternatives in a number of commercial applications. Thus adaptive growth supported by hierarchical structuring, will be a replacement for complex fabrication techniques, with the competence of constant remodeling and counteractive advantages.

***Beyond Micromachining: the potential of diatoms***

By merging the self-assembling structures of the diatoms with BaSIC for chemical versatility, synthetically processed 3-D nanostructures can be produced for use in microdevices. For example, if diatom *Coscinodiscus wailesii* is grown in the presence of nickel sulphate, then the pore size in the frustule becomes larger and this could be used in sensing applications (Figure 3.9), e.g. as an optical sensor where the change in light could be detected and information about environmental conditions be obtained.[34]

Another example is commercially available diatomaceous earth structure that could be used as a porous carbon catalyst support or current carries in fuel cells. The silica structure is templated by filling the empty space with sucrose and polymerising with sulphuric acid and carbonization in reducing atmosphere and finally removing the silica by dissolving in sodium hydroxide.[35] We can see numerous opportunities for developing new strategies which could result in multifunctional materials by hierarchical assembly, made available by biomimetic materials research.

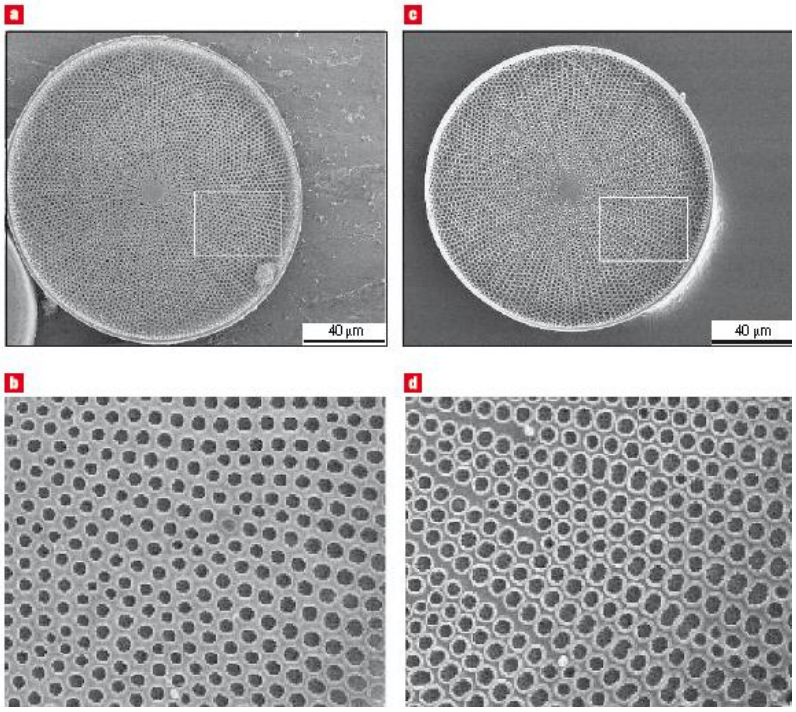


**Figure 3.9: (a,b) frustule pattern of diatom *Coscinodiscus wailesii*; (c,d) pore size becomes larger when grown in the presence of nickel sulphate** [34]

***Biomimetic materials research, lessons from the biological world:*** hierarchical structuring, growth and functional adaptation on damage repair and self-healing, provide enormous opportunities for rapidly growing and enormously promising fields. A major opportunity for bio-inspired material synthesis and adaptation into specific functions is, due to the hierarchical structuring given by a material, because of the manufacturing of both the micro-level and the nano-level structures in a single step process instead of separate processes for each of them. Pore size control ability and morphology control, along with chemical composition changeability, gives the potential to modify the structural, photonic, absorptive, diffusive and mechanical properties of the diatom frustule to outfit various applications.

Processes for the assembly of biologically fabricated microstructures into defined patterns are required for future development of device applications in optoelectronics and bio-nanotechnology. [36] Nanotechnology industry always aims to design and manufacture devices in the nanometer proportions but the current limitations for the nanotechnological devices are economy and atomic level accuracy. The nanotechnologists are attracted to the ways by which diatoms make minute silica structures – which is impossible to achieve using current materials and technology. The nanotechnological applications could be linked to atomic level filtrations, biosensors and immune-isolations or micro fabrications.[37]

For instance growing cultures of single-celled diatoms by directed assembly may provide an alternative for achieving a scale range that may not have been possible to achieve by the latest micromachining process; this shows the potential of diatoms beyond micromachining. Unanticipated discovery from the observation of nature will gradually be replaced by systematic approaches in the near future, by application of engineering principles, for further development of bio-inspired ideas. The synthesis of advanced materials having complex shapes with hierarchial architectures, is

possible by biomimetic mineralization along with size, shape and morphology control under controlled settings.

### 3.3. DIATOMS AS A NEW STANDARD FOR 3D NANOSTRUCTURE ASSEMBLY

"Biogenesis of the diatom cell wall is well thought-out to be a prototype for the controlled production of nanostructured silica".[38] In nature elegant examples exist which have intricate 3D microstructures with nanoscale features. Of these striking biological examples of self-formed rigid structures is diatoms, which are single celled micro algae with cell walls composed of amorphous silica. Diatom frustules have reproducible features in the range of micro-scale to nanoscale. Furthermore, they have nano sized assembly of silica particles assembled which could be directly utilized in a wide range of micro/nano technological applications (e.g., masks for lithographic patterning, catalyst supports, and gel filtration, so on).

Diatom nanotechnology has gained much attention because of its wide range of species, highly regular multi-scale structures, fast reproduction capabilities and also genetic manipulability. Diatoms produce a wide range of three-dimensional structures, with high rate of growth, which may be of great use in the manufacturing of components for nanotechnology, acting as an alternative to current lithographic techniques.

In other words, diatoms could be used as bio-factories to generate great number of 3D structures with identical shapes with reproducible micro-to-nanoscale features.[39] A wide range of applications is anticipated by the capability to genetically engineer diatoms to synthesize exclusive frustules.

In the face of the technological and economic promises for devices with wide variety of applications, present micro/nano fabrication methods are largely based on: layer-by-layer deposition techniques or micromachining by photolithography/chemical etching which are two dimensional (2D) in temperament. Such is not suitable for low cost mass production of 3D micro devices with intricate structures having micro/nano scale features.[40] New fabrication techniques with competent skills for yielding large volume of complex 3D structures in either silicon or non-silicon based compositions are required to be developed for wider range of device applications. For that reason diatoms surpass modern engineering capabilities, forming intricately patterned silica shells by bio-mineralizing a mixture of proteins, amorphous silica and carbohydrates.[41] Benefit is direct fabrication of 3D structures is allowed, in place of the layer-by-layer deposition technique of the present day, where lithography is used to build microelectronics.

Generally, possible approaches for using diatoms in nanotechnology are; either using naturally produced silica structures or bio-mimicking the structure as we have seen in the biomimetics section earlier. Novel micro-scale devices with meso-scale to nano-scale features for a variety of applications are in progress to development with appreciable worldwide efforts. Applications may be in a wide range such as:

- Telecommunications (e.g., optical sensors, actuators, and lenses),
- Medicines (e.g., drug delivery capsules, in-vitro sensors, membranes for chemical purification),
- Transportation (e.g., catalytic components, sensors, valves for aircraft and automobiles as catalysts),
- Manufacturing (e.g., self-assembled devices, on-line sensors, micro-robots).

At the same time, economic viability for industrial production is also mainly interested in cheap culturing so that it is more practical; this is possible with diatoms, since they just require carbon dioxide, water, inorganic salts, and light to grow the cultures. Therefore, for interested candidates, it would just need readily available fresh, sea or brackish water to make the media for cultures and then enrich it by merely adding a few organic compounds.

### 3.3.1. HIGH POTENTIAL FOR TECHNOLOGICAL APPLICATIONS

Open structure and micro to nano scale porosity of the diatom frustules has made it attractive for use in advanced micro/nanostructured devices. Diatoms have attracted the interest of nanotechnologist for the development of the discipline and practical applications. Need for precise porous structures or materials with explicit surface properties is the current requirement of the industry. Biologists and diatomists have, for a long time, studied the single-celled micro-algae together and engineers are now discovering ways and means to exploit the advantages of these organisms. Many nanoscale applications can be suggested keeping in view the unique nano-pore structures, micro-channels and mainly silica microcrystal structure.

Self-formation capabilities of these species, in silica on the micrometer scale with nanometer sized features in a wide variety of shapes and patterns create an interest in the diatom development. These shapes can be recreated, under different topological combinations, and glued to substrates to create nano-substrates and nano-patterned nanomaterials or in other applications of nanotechnology.(42) Angularity, rigidity, and inertness not available in other microbes give the diatoms inimitability in using the complex 3D arrays of silica pores for sorting of particles and nano-fluidics because the molecule size that can be altered over the paths are tens of nanometers in size and in more such kinds of applications.

For example, diatom frustules can be embedded into a metal-film membrane, for pinpoint drug delivery by magnetizing the diatoms or silica nano-powders could be produced.(43) Thus many applications of diatoms are introduced into our day-to-day life. For instance, Native Americans used Diatomaceous Earth (DE)[1] to protect their crop from insects and ants by physical injury rather than to chemically poison. So, we can say that there are several reasons for the diatoms to become interesting for the micro/nano-technological applications in specific, such as:

***Size, Morphological Diversity, and Complexity.*** Because of the µm to mm sizes, light weight and stability, and shapes such as cylindrical, ellipsoid, cubic or needle-shape, all together increase the chances of using diatoms in technological applications. Using the frustules directly or in a slightly modified form is easy. Thus diatom frustules with their diverse geometries and pore sizes present a wide selection of attributes that the micro/nano-engineering can exploit.

***Motility.*** Considering the diatom movement when attached to a surface, this could perhaps lead to usage of patterned surfaces for guiding them into particular places, by phototrophism or geotropism, where they could grow to be nanotechnology components. For which we can attract them by either using organic or inorganic materials such as light, nutrients, etc.

***Material and Structure formation.*** Amorphous silica is used by the diatoms, which makes it possible to cast their shell material in any form of shape in the silica deposition vesicles (SDVs) inside its body, making it easy to build very smooth and statistically better-quality structures. As a result the frustules (rigid walls) of the diatoms consist of intricate 3D structures constituting of

[1] Diatomaceous Earth : a heterogeneous mixture of fossilized remains of diatoms

micro-scale pores and channels. This combination of silica chemistry on a high area is suitable for applications such as microscale total analysis system.

The diatom frustule may perhaps be chemically modified for antibodies attachment, possibly be used in immunoprecipitation; exceptionally cheap and renewable material process, produced using only light and minimal nutrients.[44] Therefore, a detailed understanding of the diatom frustules from the nano scale level to the entire shell level may provide insights for; advanced combinations of nanostructured ceramic materials and light weight architectures for technological applications, because of their high degree of symmetry and complexity. We also have a technique where, only symmetrical diatoms could be selected by "compustat selection" experiments (i.e. forced evolution) which has been suggested be Robert Gordon.[45] [46] Here only diatoms having the same kind of structure, size and other features specified by the user are matched and selcted while the others are killed to be thrown out of the culture under analysis.

Making complex nanoscale three-dimensional structures at low cost and in large numbers is the key to the development of nanotechnology. A wide variety of structures in the silicified cell walls offers a great scope, where diatom silica can be converted into other materials by maintaining the nano-scale morphology, which upon desired specific *in vivo* manipulations determines use in nanotechnology. By introducing modified genes into the diatoms i.e. straightforward gene modification is possible but currently replacing the native diatom gene with modified copies is a problem.[47] [48] Hence, by molecular genetics techniques, diatom silicified structures can be modified according to the requisite and used for specific distinctive applications.

Furthermore, another useful property is the ability to dope the biosilica. Certain chemicals can also be incorporated into the cell's frustule by adding to the culture medium or biologically via cell's silica transporters. So, the transparent nature in water due to its refractive index can be modified by incorporating the dyes and making it much easier for handling. On the whole, the advantages presented by diatom frustules cannot be surpassed by any of the current technologies available for micro/nanomanufacutring, and is thus appealing for the technological applications.

### 3.3.2. TECHNOLOGICAL APPLICATIONS

The highly ordered 3D porous silica structures hold a promising vicinity for the biological or biomimetic fabrication of nanostructured devices and materials. Diatoms are relatively new sources of inspiration for design and fabrication of nano-structured materials where inorganic structures are synthesized with ordered micro-to-nanoscale features. The frustules of the diatoms are decorated with nano-sized features such as pores, ridges, spikes and spines, composed in amorphous silica in a single-cell. So these have been used for a considerable number of experiments such as recovery of metals from waste streams, gas or waste water purification, filtration of beverages, agricultural addictives also as porous partition columns for chemical analysis, a few among these could be seen.

#### 3.3.2.1. CHEMICAL REDUCTION OF SELF ASSEMBLED SILICA STRUCTURES - FOR GAS SENSORS

Ability to convert diatom frustules into semiconducting ceramics like $TiO_2$, $GeO_2$, $ZrO_2$, or $SnO_2$ by methods such as BaSIC, sol-gel chemistry, hydrothermal conversion or bioengineering gives scope for gas-sensing applications. Depending upon the morphology and porosity of the frustule, the diatoms usually exhibit a variety of relative responses and varied gas concentration ranges of sensitivity.

First the diatom replica is molded onto a soft and elastic polymer using poly(dimethylsiloxane) PDMS as a master; this is then used as a mold to fabricate the actual structures of the diatoms in either polymers or ceramics or any other material. This demonstrates the use of diatoms for the rapid and simple fabrication of polymer nanostructures. Several hierarchical structures in the frustules with different feature sizes and patterns are copied. A wide range of potential applications of such structures include optics, photonics, catalysis, bio-sensing, drug delivery, filtration, bio-encapsulations and immunizations. The benefit is that diatoms are cheap resources and moreover remarkable number of masters exists with a range of structures and patterns across the micro- and nanoscale.(49)

We also have another alternative, where three-dimensional nanostructured silica micro-assemblies formed by the diatoms can be converted into nanocrystalline silica replicas. A magnesiothermic reduction process at a tempreature of roughly 650°C is used for converting; then, the structures are converted in to co-continuous, nanocrystalline mixture of slicon and magnesia by reaction with magnesium gas. These formed silicon replicas are photolumiscent and exhibited raphid changes in the impedance when exposed in gaseous nitric oxide (a possible application in microscale gas sensing).

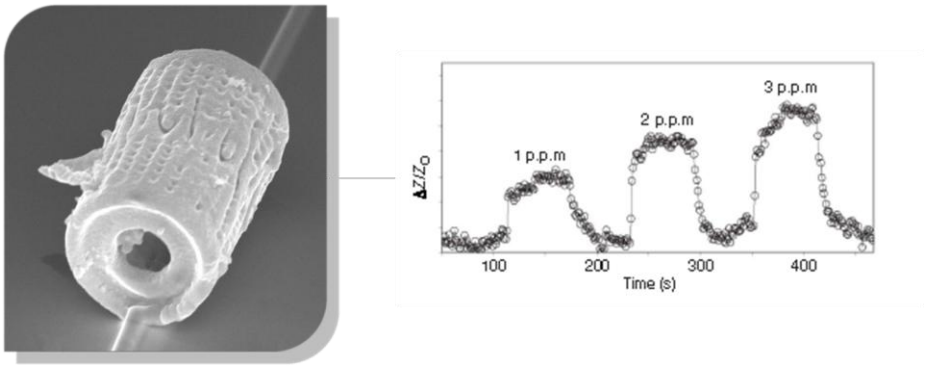


**Figure 3.10: Nanocrystalline silicone senor made by reduction of diatom silica structure using magnesium oxide for sensing $NO_x$ gas (left) and electrical response of the frustule to NO(g) (right)** (50)

A unique and explicit behaviour of the diatoms is the formation of nanoporous biosilica inside the body, by collecting 'Si' from the water to form several micrometers to several hundreds of micrometers of biosilica, containing nanometer to several hundred nanometer sized nanopores. These nanoporous silica known as frustules can be used in filters, carriers, support for chromatography, and building materials. Either synthetic silica templates for sensors, electronics, optical or biomedical applications can be made easily. (50)(51)

#### 3.3.2.2. HIERARCHICAL STRUCTURED DIATOMITE – FOR THE REMOVAL OF COBALT IONS

Unicellular diatom cells are usually enclosed in hydrated silica cages, with cell walls having 3-dimensional distribution of pores. Surface topography of these diatoms may be of use for the sorting and filtering of particles of nano-to-micro size range. With respect to their complex shapes, having a large surface area gives them excellent absorption properties. These diatoms are of use not only when alive, but also after they die, their sediments are of great use still. The frustules accumulate and condense at the bottom of the water bodies, after the death of the diatoms and in suitable condition they form a considerable thickness, known as diatomaceous earth (diatomite).

Accordingly, for the removal of cobalt ions such a diatomite is used. Diatoms prove to be good support for zeolites because of the macroporous structure useful for filtering and also a cheap raw material for application. A hierarchical porous material is synthesized by hydrothermal growth of faujasite (a mixture of different minerals) on diatomite frustule surface (Figure 3.11), which is earlier seeded with nano-zeolite crystals. Zeolites are seeded into the diatoms for ion exchange and catalysis, so that it can remove cobalt ions, both of which are common components of radioactive aqueous waste.[52]

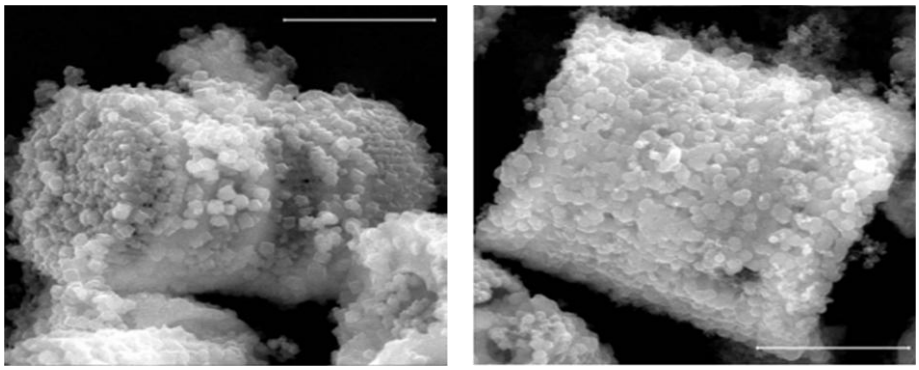

**Figure 3.11: Diatomite with faujasite crystals grown on the surface [52] (scale bare = 10 µm)**

The new material synthesized by zeolitization of diatoms removes 2.4 times amount of ions than the normal zeolite. Therefore, stabilizing the nuclear waste into solid form can save expensive monitoring and hazardous harms. Thus, diatomaceous earth as filter will encapsulate contaminants both in particulate and ionic forms, because of its properties with combination of zeolitie's ion exchange, allowing long term of handling. Consequently this could turn out to be a promising alternative for the removal of many such hazardous contaminants.

#### 3.3.2.3. CONDUCTING DIATOMITE FILLERS

A conducting diatomite is made by polyaniline on surface modification of the diatomite. Diatomite itself is usually used as filler in paints, paper and rubber because of its properties; it is insoluble in water and does not respond with other substances in air, and it acts as an insulator and non-inflammable. Advantages are also that it is light weight, porous, stable and less expensive filler. But, polyaniline diatomite composite is much more useful because, the applied fields of insulation by the diatom are enlarged here making it much attractive for the industrial application. (Polyaniline itself is a great conducting polymer with easy preparation and excellent environmental stability)

Figure 3.12(b) shows that many polyaniline particles filled pores of the normal diatomite in Figure 3.12(a). Although it contains only 8% conducting polyaniline by mass, the conductivity increased to $2.8 \times 10^{-2}$ S $cm^{-1}$ at 20°C. Also, the thermal degradation temperature of the conducting diatomite is 390°C in air which is lower than pure polyaniline.[53][54]

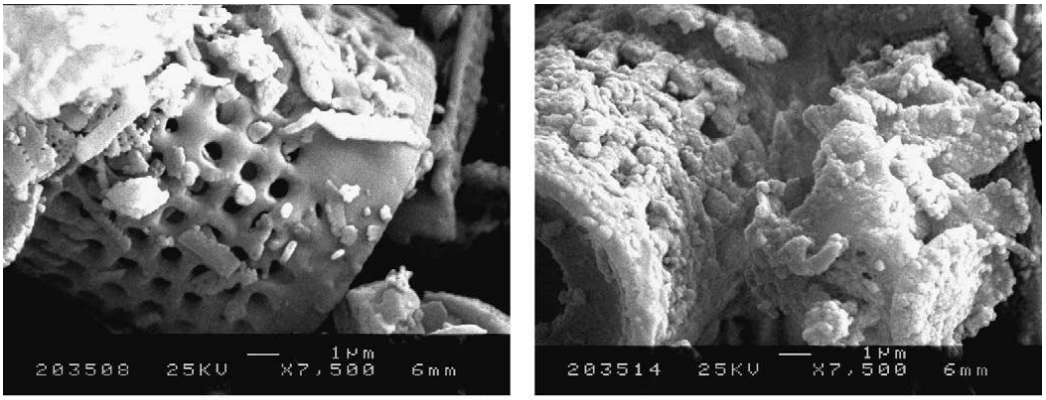


**Figure 3.12: SEM of (a) normal diatomite (b) conducting diatomite** [53]

Finally, this polyaniline coated diatomite has a potential commercial appliance in conductive coating and also electromagnetic shielding materials. It is important to note that polyaniline does not easily blend with diatomite and only an interaction occurs at the surface of the diatomite; this small reaction also gives high-quality consequences. Accordingly a detailed research on more methods would prove useful in many more applications.

### 3.3.2.4. DIATOM REPLICAS IN PHOTONIC DEVICES

Diatoms have intricate geometric structures and spectacular patterns in the silica-based cell walls, which is why they are so efficient at photosynthesis. The micro diameters and nano scale thickness of the diatom frustules in hexagonal patterns of holes could act as photonic-crystal slabs; having the capability of confining photons coupled into the slabs is the reason for high efficiency in photosynthesis. Thus diatom cells could act as natural mini-photonic devices, with competence in collecting and controlling light cleverly. But the diatom bio-silica may not give the optimum chemical/refractive index appropriate for many applications, so altering their chemistry could offer several advantages over current technologies. Therefore, by a sonochemical process, replicas are produced of metal chalcogenide (ZnS) meso/nanostructures. Chalcogenides because they commonly posses a high refractive index, useful in optics and photonics. [55]

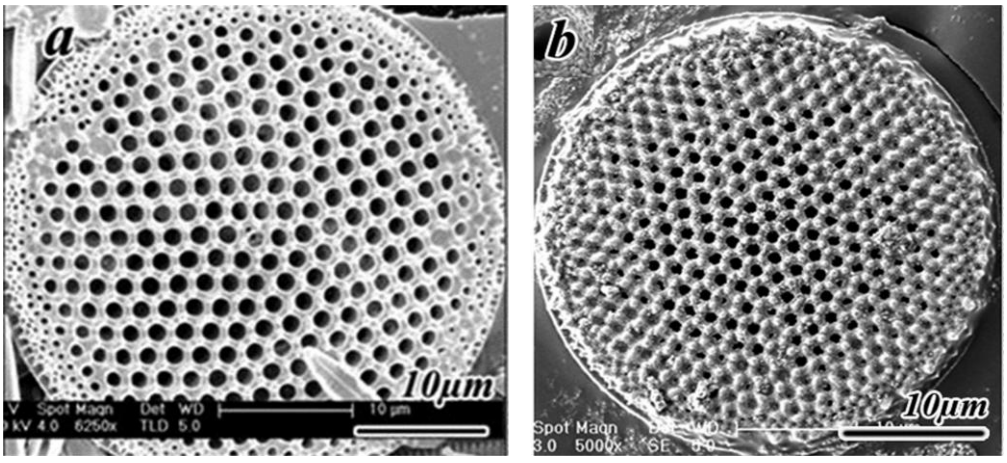


**Figure 3.13: (a) Frustule structure of diatom *Coscinodiscus lineatus* (b) ZnS covered frustule replica**[55]

By the sonochemical process, ZnS particles are deposited on the diatom frustule structure. Into a suspension of diluted diatom culture (*Coscinodiscus lineatus* (figure 3.13(a)) Zinc acetate and

thioacetamide are dispersed. By an ultrasonic cleaner, the solution is sonicated for 3 hrs at room temperature. Filtered and washed with distilled water and ethanol, ZnS covered frustules (figure 3.13(b)) are obtained which have higher refractive indices than the natural frustules.

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# *Chapter 4*

## 4. STAMPS

### *Microstructure for orientation and immobilization of Diatoms*

For the orientation and immobilization of the diatoms we designed a PDMS stamp for printing of organic and inorganic materials on glass substrate and a patterned glass plate for light illumination. The diatoms are attracted by light and the assumption is that they will creep towards the light and reside on the illuminated spot.

There are two versions of the pattern for the illumination, one with a single hole structure and dimension as the stamp and one with two smaller holes in the same pattern as the stamp. The glass plate should be covered with Titanium that is thick enough to be non transparent for light. The typical chromium mask is about 100 nm. As titanium is oxidized the thickness of the titanium should be increased to about 200 nm.

Soft lithography has been employed to fabricate the masters. A patterned elastomer is used as a mask, stamp or mold. Rapid prototyping procedure is followed for soft lithography. The pattern is composed and transferred to a CAD file and printed on a transparent sheet of polymer using a commercial image setter. The patterned sheet is used in contact photolithography to prepare a master in a thin film of photo resist. The negative replica of this master in elastomer becomes our require stamp or mold. It is a very easy and cost effective procedure; tome from the design to stamp fabrication takes less than 24 hours on the overall.[56] Soft lithography also offers the ability to control the molecular structures on the surfaces and pattern the complex structures compatible with biology.[57]

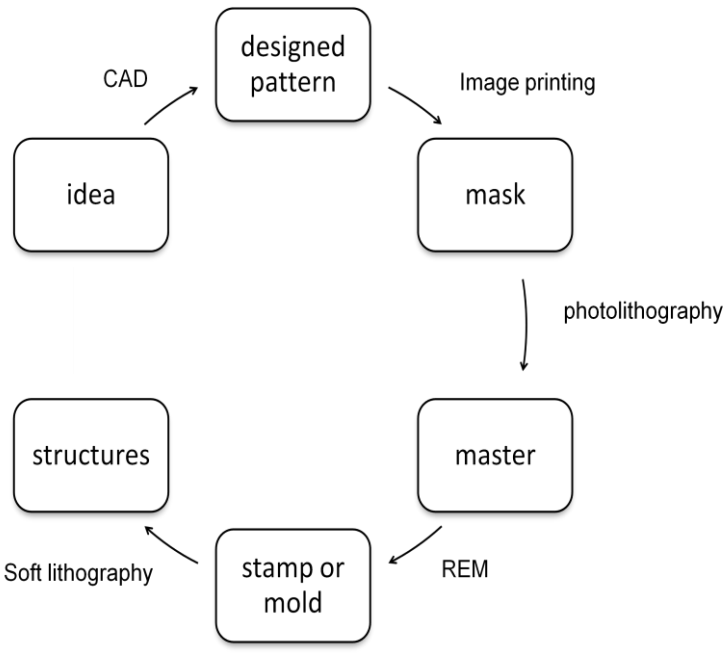


**Figure 4.1: General procedure for soft lithography** [56]

The light illumination pattern is deposited on 0,5mm thick and 100mm diameter Borofloat wafers. The pattern area is 5x5 mm and cut into pieces that are 15 x 15 mm wide.

The Master size is 5x5 mm, with circular holes 20 μm wide and 30 μm deep. The space between adjacent holes is 200 μm in total 25 x 25 holes. Design is a compromise between the height of the pillar that should be large enough to get a good imprint and low enough so the stamp could be released from the master. The aspect ratio is 1.5 which is within the design rules for soft lithography.

The CAD layouts (Appendix – I) are prepared by Swerea IVF and adopted by Tekniker for processing.

## 4.1. SILICON MASTER FABRICATION

### 4.1.1. PROCESS FLOW

Silicon wafers (diameter: 4 inch, thickness: 525 μm) were dehydrated at 180ºC for 30 min. in a forced ventilation oven. HMDS (hexamethyldisilazane, ABCR GmbH) adhesion promoter and subsequently ma-P 1275 (Micro Resist Technology GmbH) positive photo resist were spin coated at 3000 rpm for 30 s.

The coated silicon wafers were soft-baked in a hot-plate for 300 s at 100ºC. The photo resist was exposed in an EVG 620 mask aligner, using a chromium-on-glass photolithographic mask (Figure 4.2) designed in Tekniker according the required specifications (from Per Johander at Swerea IVF) and processed by ML&C GmbH. The exposition time was 3 s and the light intensity was 10 mW/cm2, at a wavelength of 350-450 nm.

The photo resist was developed by immersion of the wafers in a ma-D 331 (Micro Resist Technology GmbH) for ca. 180 s. Then the wafers were immersed in deionized water to stop development and rinsed with more deionized water to remove remaining developer.

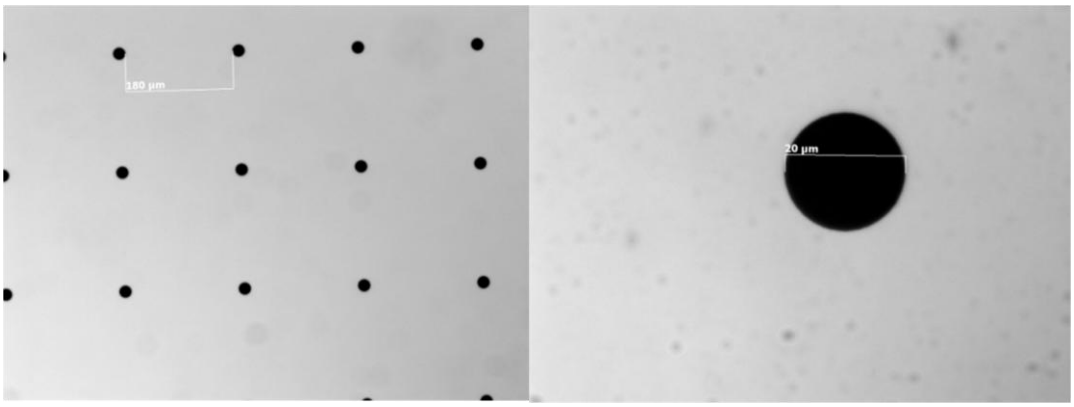


**Figure 4.2: Details of photolithographic mask. White areas correspond to chromium and black areas to glass.**

Developed areas of the photo resist make silicon available to etching. The etching was carried out in a Plasmalab 80 reactive ion etching system, using a Bosch process. This process uses alternative steps of etching (with $SF_6$) and passivation (with $C_4F_8$) in order to obtain an anisotropic etching. Tests (Figure 4.3) were carried out in order to obtain the number of steps necessary to etch 30 μm in depth. The parameters used in the obtained silicon masters appear in Table I.

| | |
|---|---|
| High current steps | **5** |
| Low current steps | **25** |
| Gas flow ($SF_6$ steps/$C_4F_8$ steps) | **120/100 sccm** |

| | |
|---|---|
| RF power high current ($SF_6$/ $C_4F_8$) | **30/10 W** |
| RF power low current ($SF_6$/ $C_4F_8$) | **15/5 W** |
| ICP power | **300 W** |
| Etch/passivation times | **10/5 s (high current); 11/4 s (low current)** |

**Table I: Bosch process parameters**

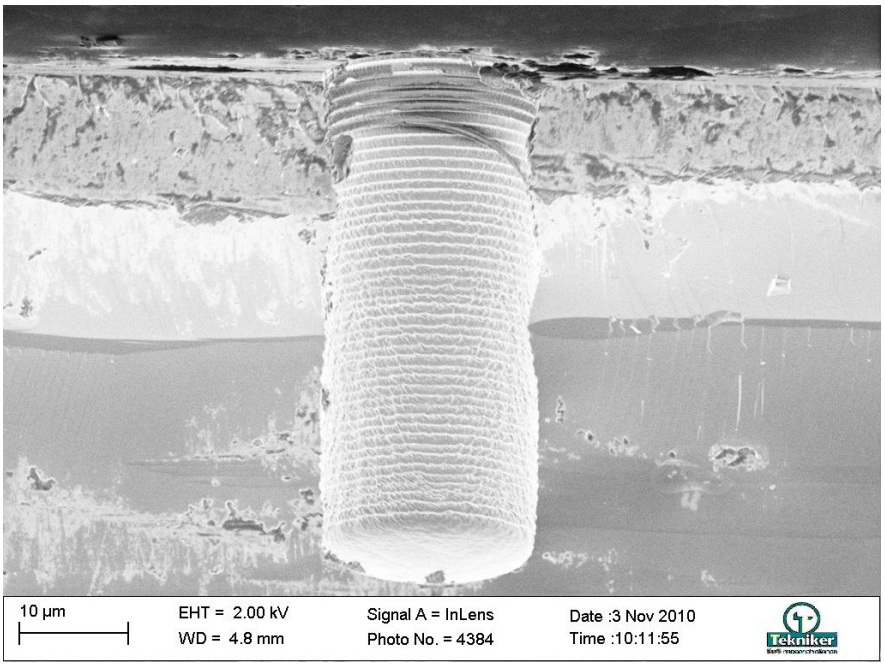


**Figure 4.3: SEM photograph of a test etching carried out with a Bosch process with 35 alternative steps of $SF_6/C_4F_8$. This test gave rise to a depth of 45 µm**

After the etching, the structured wafers were cleaned using acetone, isopropyl alcohol and deionized water and diced to obtain individual silicon stamps (15 x 15 mm) in a Disco DAD 321 Dicer. Each dice wafer contains a patterned area of 5 x 5 mm, where there are nearly 484 holes, separated by a distance of 200 µm, having a diameter of 20 µm (approximately) (Figure 4.4, (i) showing a part of the pattern (ii) distance between 2 adjacent holes (iii) diameter).

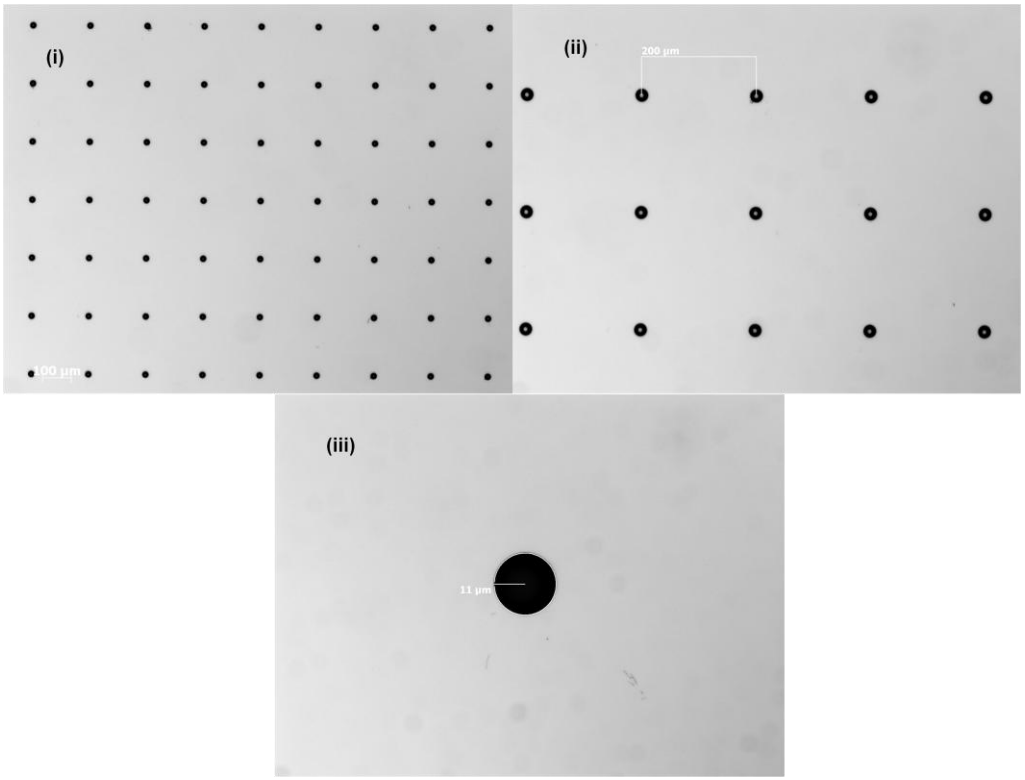


**Figure 4.4: Details of silicon stamps. (i) a part of the pattern showing few holes (ii) distance between 2 adjacent hole ~200 µm (iii) diameter of single hole ~20 µm**

After dicing, the stamps were cleaned again, and characterized using an interferometry microscope (Wyko NT 1000) and light microscopy. The characterization reports are as below (Figure 4.5 and Figure 4.6).

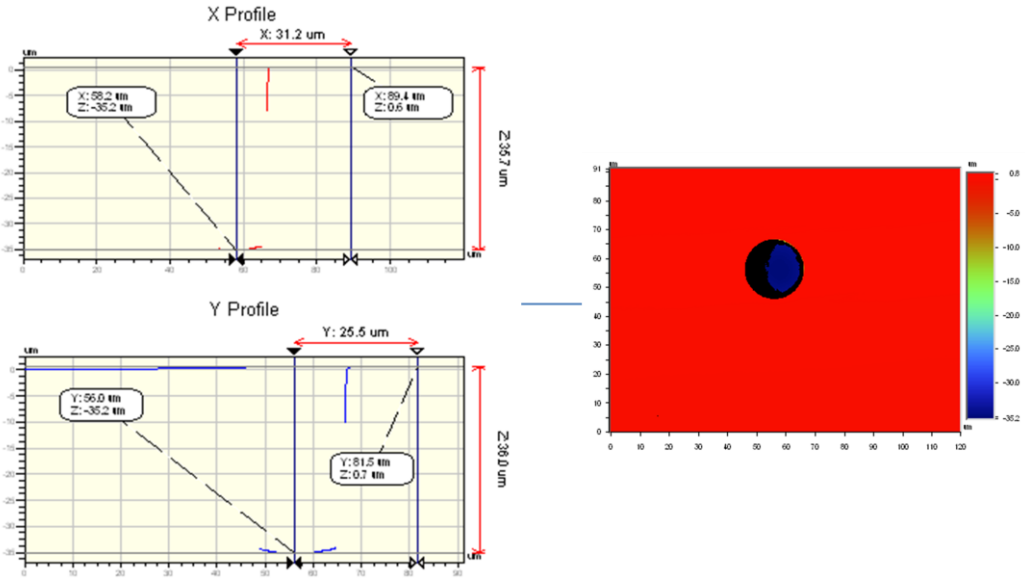


**FIGURE 4.5: Light microscopy characteristics of a hole in the center of the silicon wafer. Depth obtained is ~ 35 µm**

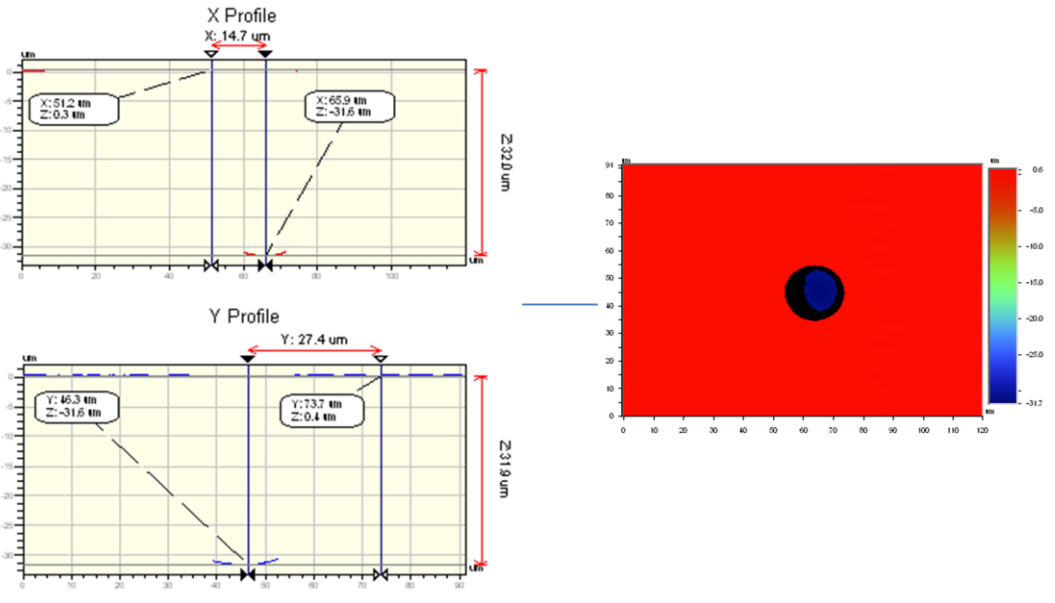


**FIGURE 4.6: Light microscopy characteristics of a hole in the edge of the silicon wafer. Depth obtained is ~ 30 µm**

In the silicon stamp, there are 22x22=484 circumferences (Ø20 µm separated by 200 µm). We have done Scanning Electron Microscopy (SEM) to look at an entire array of hole in a single view (Figure 4.7). Each hole is 30 µm deep; this stamp is further used for making PDMS stamps, where we can get columns of this height.

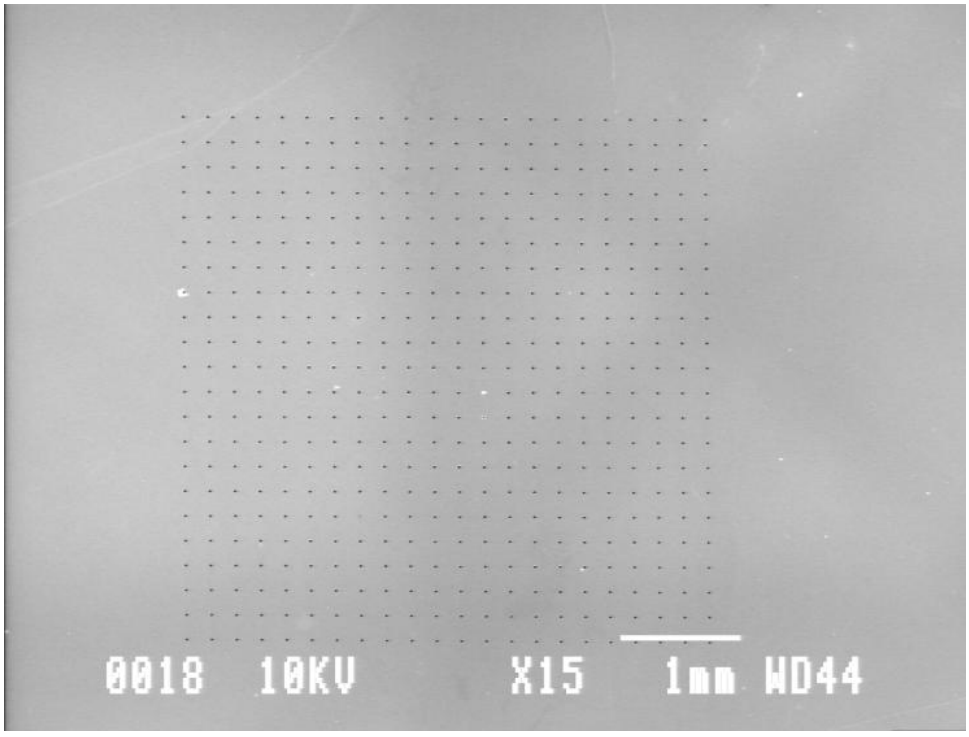


**FIGURE 4.7: SEM at 15X showing an entire array of holes on a silicon wafer**

### 4.1.2. PDMS CASTING

PDMS (Poly (dimethylsiloxane)) stamps for printing of organic and inorganic materials on glass substrate are manufactured at SwereaIVF, using the silicon stamps having holes of 20 µm wide

separated by 200 μm (made by Tekniker). The procedure followed for the PDMS fabrication is as below, where first an antiadhesive layer is coated on the silicon stamp and then the PDMS moulds are fabricated.

Antiadhesive layer: In order to demould the PDMS without problems it is necessary (carried out a test without silanizing agent and there was ripping off of the structures), to use a silanizing agent. We can silanize a number of silicon masters at the same time. A vacuum dessicator connected to a vacuum pump is used for the silanization. At the bottom of the dessicator a few drops (which is enough to cover the bottom) of the FOTS (1H,1H,2H,2H-perfluorooctyltrichlorosilane) are kept in a small Petri dish. Above, in a Petri dish a number of silicon masters are placed, a space is left between the bottom dish and the upper dish so that the FOTS can evaporate and fill all the space in the chamber. The FOTS is left to evaporate in the chamber at vacuum during 30 min-1hr. Then the excess of FOTS is washed-off using hexane (then blow the hexane to dry the stamp).

PDMS moulding: The bottom of a Petri dish was covered with aluminum foil (to simplify separation of the silicon master from the Petri dish if there was an escape from the frame). Silicon masters are placed in the Petri dish. A frame is cut from a silicone sheet in order to avoid the PDMS flowing (PDMS frame with size smaller than that of the silicon master). To have a better adhesion between the PDMS frame and the silicon, the PDMS frame is wet (a little) in ethanol before being put into contact with the silicon.

***Typical Experimental Sequence:***

Weigh the PDMS base (Sylgard 184 big container) in a glass container and add the initiator (small bottle) in a ratio 10:1 (PDMS base: initiator). Approximately 1-2 g per stamp is used. Stir a bit the mixture using a spatula, and pour the mixture over the silicon masters limited by the PDMS frames. Put the Petri dish with the silicon masters covered by the PDMS into a dessicator connected to a vacuum pump or into a vacuum chamber for at least 1 hour (longer time, less bubbles, better reproduction fidelity) in order to eliminate the bubbles that are in the cavities and that don't let the PDMS fill the holes. After this period, silicon masters covered by the PDMS are placed into an oven at 100ºC for 1 hour. Then the masters are left to cool down and finally the PDMS stamps are carefully peeled off.

Characterizations of the height of the pillars were done in order to verify that the heights obtained are the same as the depth of the holes in the silicon stamps. Interferometer measurements showing the heights of the pillars obtained in the PDMS stamps (Figure 4.8). The pillars fabricated on the PDMS were observed in an optical microscope (Figure 4.9). Height of the pillars was measured and this confirmed that all the pillars on a single PDMS were of the same height i.e. ~ 31 μm.

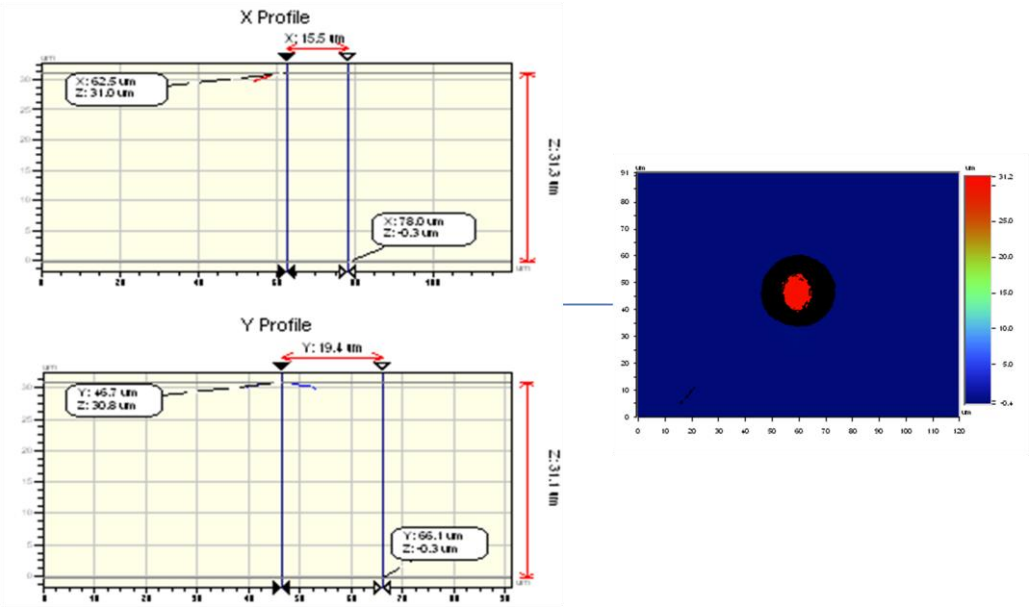


**FIGURE 4.8: Light microscopy characteristics of a pillar on a PDMS. Height obtained is ~ 31 µm**

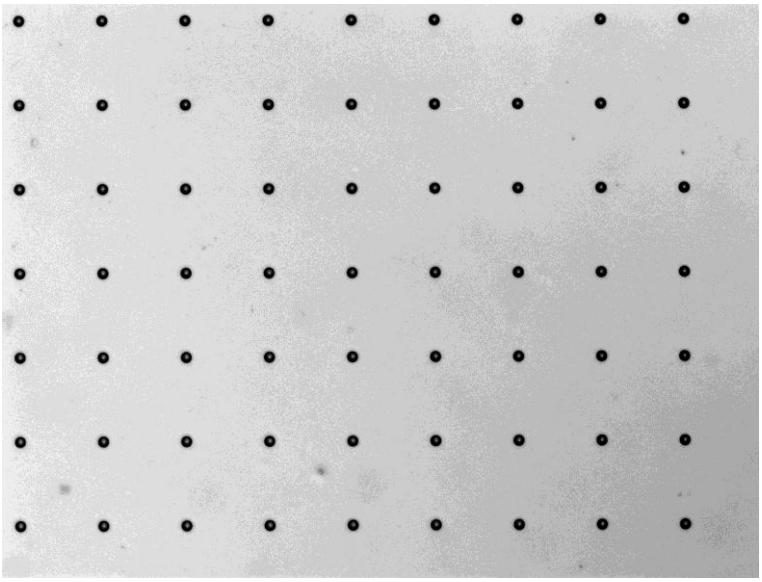

**Figure 4.9: Optical microscopy image of PDMS stamp with pillars. A small bright spot in the center of each big dark spot represents the top of the pillar.**

To get a clear view of the pillars formed on the PDMS, we did scanning electron microscopy on the PDMS stamps after coating them with carbon, to reduce the conduction in the SEM. In Figure 4.10 you can see the SEM images of the PDMS, the entire array as per the first CAD layouts, and to get a clear view an enlarged view of the pattern is taken. All the pillars are of the same height and this is good, so the stamping of organic or inorganic material on to the glass slides will be uniform.

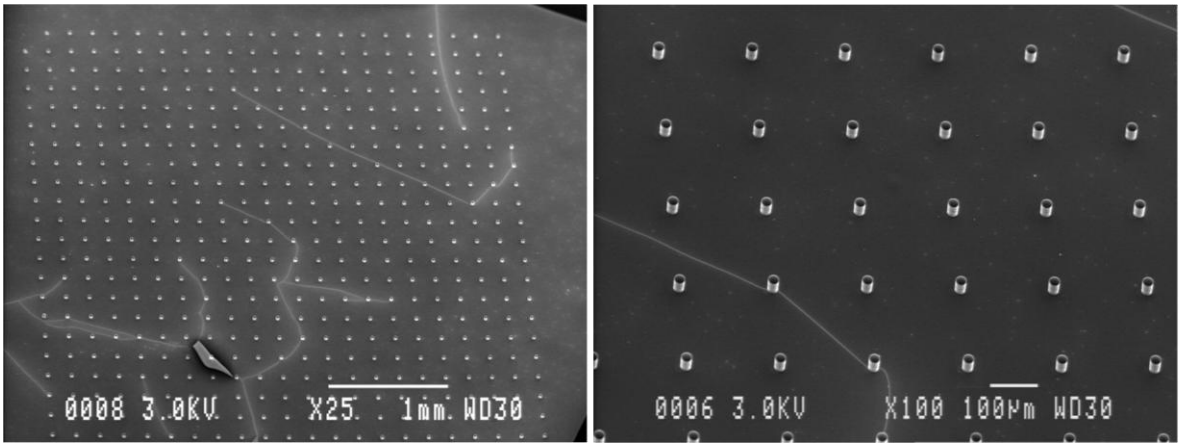


**Figure 4.10: SEM images of the pillars on the PDMS stamp**

### 4.2. TITANIUM-ON-GLASS MASTERS FABRICATION

***A.*** For the fabrication of 20 µm diameter holes in titanium over a glass substrate, 4" diameter Pyrex 7740 wafers were dehydrated in an oven at 180ºC for 30 min. Then, ma-P 1205 positive photoresist (MicroResist Technology GmbH) was spin-coated at 3000 rpm, giving rise to a layer of ca. 500 nm. Soft-baking of the wafer was carried out in a hot-plate at 105ºC for 60 s in order to eliminate the remaining solvent.

Ultraviolet lithography was carried out in an EVG 620 mask-aligner using a mask designed for this task:

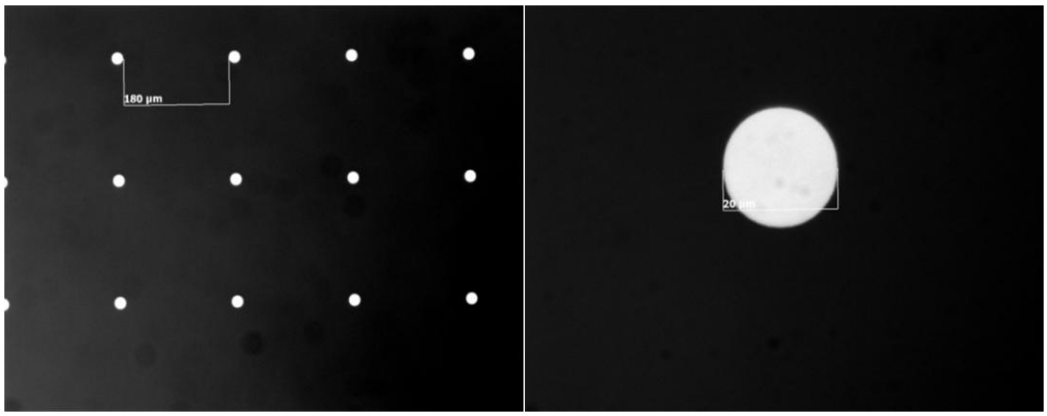


**Figure 4.11: Details of the photomask used in the UV photolithography**

An exposure time of 3 s with an intensity of 10 mW/cm$^2$ was used, using vacuum contact between the mask and the resist. Development of the resist was carried out using maD-331 developer (MicroResist Technology GmbH). This process led to large areas of glass and to pillars of photoresist with 20 µm diameter.

Titanium was deposited in a physical vapor deposition system via sputter deposition (Millenium Coatings). The wafer was first subjected to a glow discharge cleaning with a RF source at 13.56 kHz with a power of 200 W for 2 minutes in an atmosphere of Ar at 10 mTorr. The deposition was carried out using a solid titanium target (99.99% purity) and a pulsed source with a power of 1500 W for 170 s under a vacuum pressure of $5.10^{-7}$ Torr. The deposited layer had a depth of 170-190 nm. Lift-off of the remaining photoresist was carried out using acetone in an ultrasonic bath at 40ºC. The lift-off process led to the solution of the remaining photoresist but also to the elimination of the

titanium deposited over the photoresist, giving rise to titanium covered glass with holes of the desired dimensions.

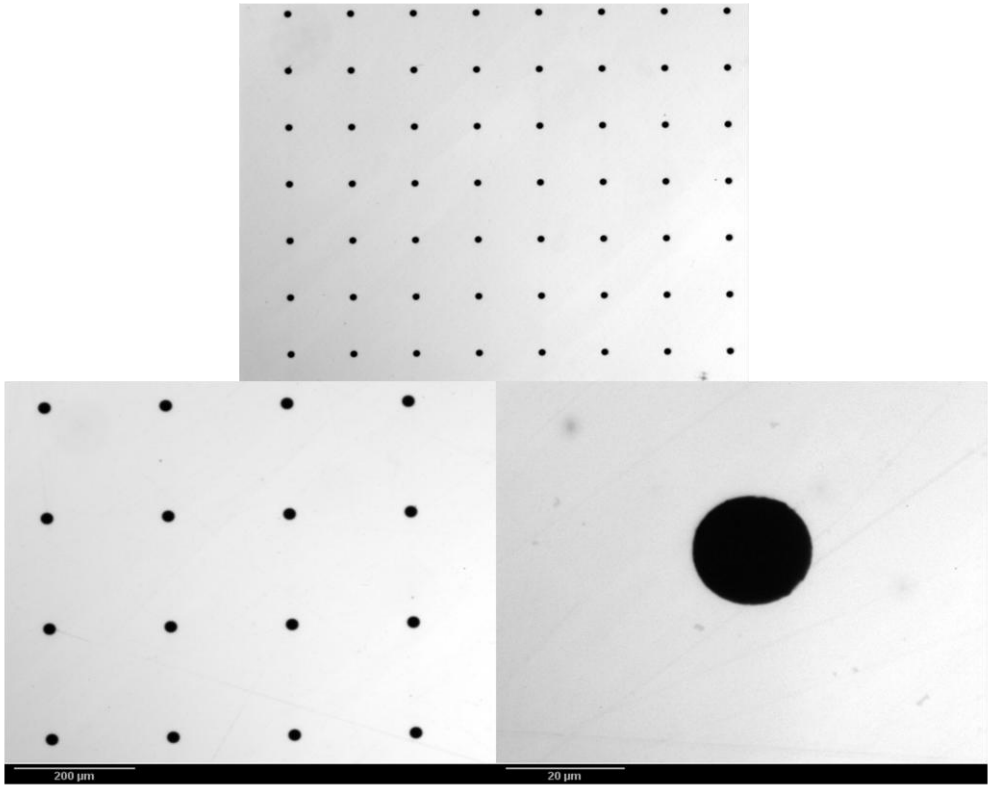


**Figure 4.12: Array of 20 µm holes in titanium and details showing the dimensions**

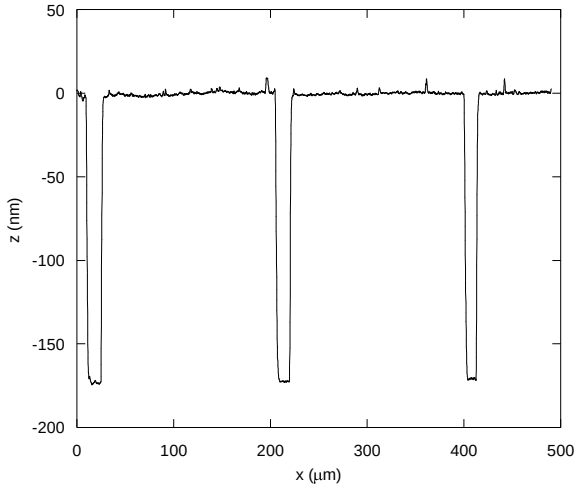


**Figure 4.13: Depth measurement in a Veeco Dektak 8 contact profilometer**

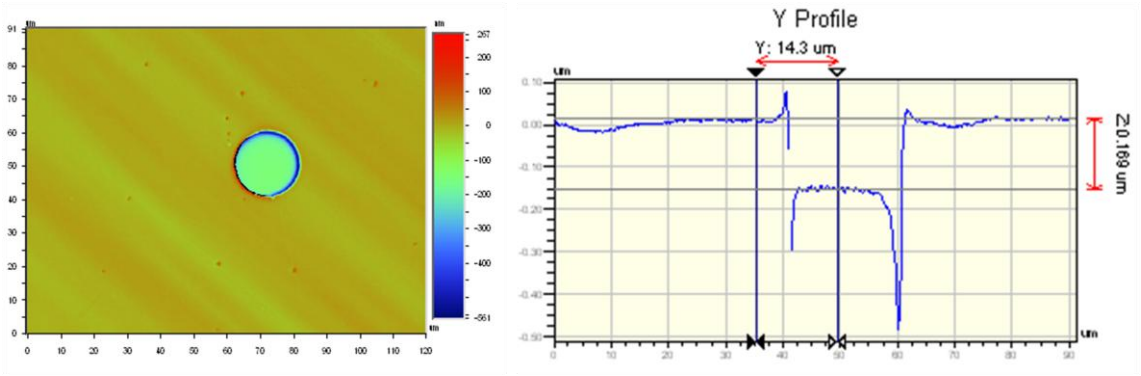


**Figure 4.14: Depth measurement in a Veeco Dektak 8 contact profilometer**

Finally, the wafers were cut to final desired size (15 mm x 15 mm squares) using a Disco DAD321 automatic dicing system.

***B.*** The method described before led to incomplete lift-off of the 3 μm structures. For the fabrication of 3 diameter holes in titanium over a glass substrate 4" diameter Pyrex 7740 wafers were dehydrated in an oven at 180ºC for 30 min. Then, ma-P 1205 positive photoresist (MicroResist Technology GmbH) was spin-coated at **1000 rpm**, giving rise to a layer of ca. **900 nm**. Soft-baking of the wafer was carried out in a hot-plate at 105ºC for 60 s in order to eliminate the remaining solvent.

Ultraviolet lithography was carried out in an EVG 620 mask-aligner using a mask designed for this task:

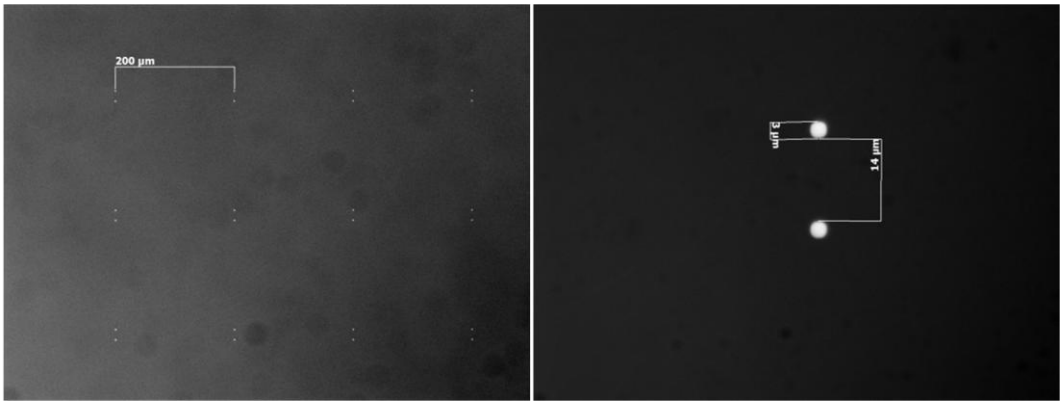


**Figure 4.15: Details of the photomask used in the UV photolithography**

An exposure time of 3 s with an intensity of 10 mW/cm$^2$ was used, using vacuum contact between the mask and the resist. Development of the resist was carried out using maD-331 developer (MicroResist Technology GmbH). This process led to large areas of glass and to pillars of photoresist with 3 μm diameter.

Titanium was deposited using an electron beam physical vapor deposition system (AJA International) which leads to a more vertical deposition than the sputtering method. The wafer was first subjected to a glow discharge cleaning, and then, titanium pellets (99,99% purity) were evaporated using the e-beam with a voltage of 7,2 kV and an emission current of 120mA, under a vacuum pressure of $1.10^{-8}$ Torr. The deposition rate was 1 Å/s. The deposited layer had also depth of 170 nm (the system has a quartz crystal system that allows online measurement of the thickness). Lift-off of the remaining photoresist was carried out using acetone in an ultrasonic bath at 40ºC. The lift-off process led to the solution of the remaining photoresist but also to the elimination of the

titanium deposited over the photoresist, giving rise to titanium covered glass with holes of the desired dimensions.

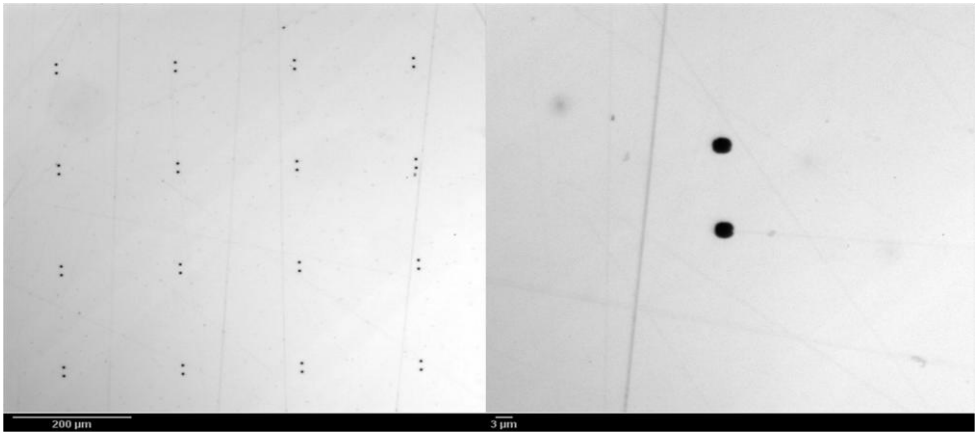


**Figure 4.16: Array of 3 µm holes in titanium and details showing the dimensions**

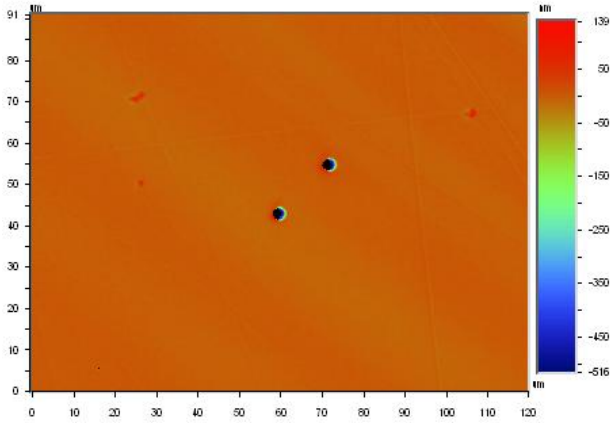


**Figure 4.17: Interferometry image of 3 µm holes (the reduced dimension of the holes did not allow us to measure the depth, the tip of the profilometer could not either reach the bottom of the holes)**

For a maximum view of the pattern scanning electron microscopy is carried out for the 20 µm holes (Figure 4.18) and 3 µm holes (Figure 4.19).

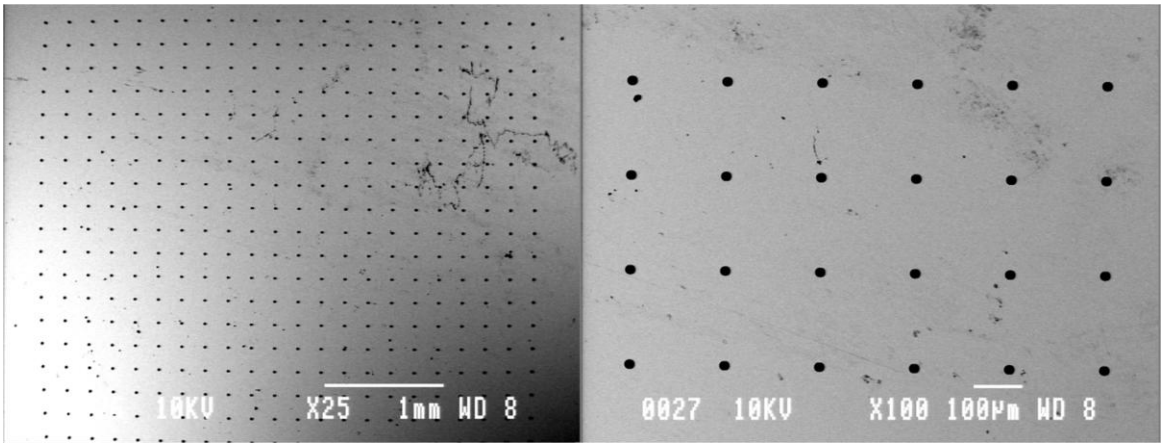


**Figure 4.18: SEM showing single hole of 20 µm in Titanium-on-glass masks**

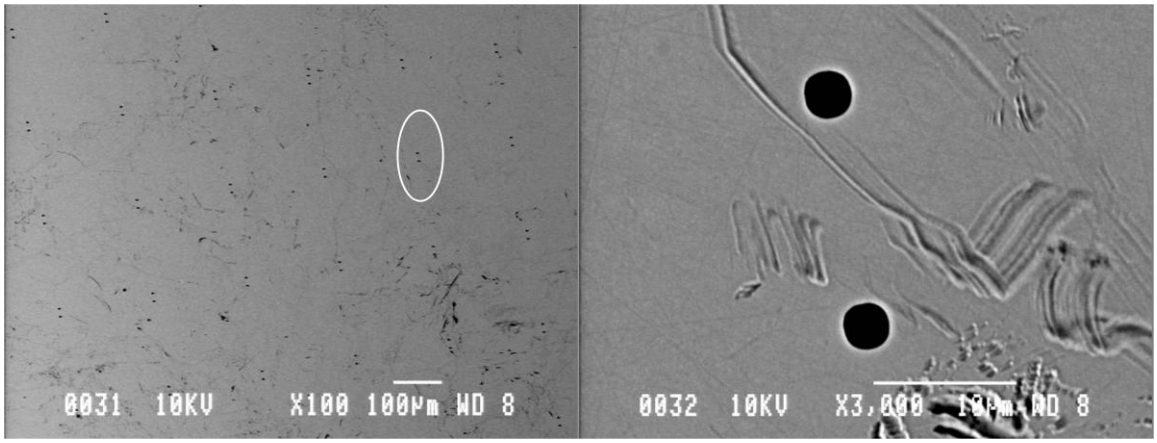


**Figure 4.19: SEM showing two holes pattern of 3 µm in Titanium-on-glass masks**

*Chapter 5*

# 5. RESULTS

## 5.1. SEM ANALYSIS OF *SURIRELLA* DIATOMS

For a clear view of the *Surirella* diatom frustule and its structure at a minute scale, SEM analysis is carried out. It is necessary to clean the diatoms in such a way that it gets rid of the biological contents and also the nutrition's present in the culture media. There are different methods to clean the diatoms, some are quick and dirty and others such as acid rinsed method to give much enhanced clear diatoms.

### 5.1.1.1. RECEIPES TO CLEAN THE DIATOMS

***Quick and dirty:***

The culture is filtered onto a 1 µm Nuclepore polycarbonate filter and quickly rinsed with distilled water.

***Acid rinsed samples:***

Culture samples are treated with 30% Sulphuric acid ($H_2SO_4$) and saturated potassium permanganate ($KMnO_4$), and left for 3 days. Oxalic acid ($(COOH)_2$) is added until the sample turns transparent. The sample has to be subsequently rinsed several times with distilled water.

### 5.1.1.2. OBSERVATIONS IN SCANNING ELECTRON MICROSCOPY

For scanning electron microscopy a drop of the acid rinsed sample is placed on a glass cover-slip, left to dry, and adhered to a stub. The stubs were then sputter coated with approximately 15 -25 nm gold using a SEM Coating Unit E5000, *POLARON EQUIPMENT LIMITED* and examined in a scanning electron microscope, model JSM-840A. Figure 5.1 shows *Surirella* on a glass cover-slip, it is difficult to view the inner structures and the structure is also unclear.

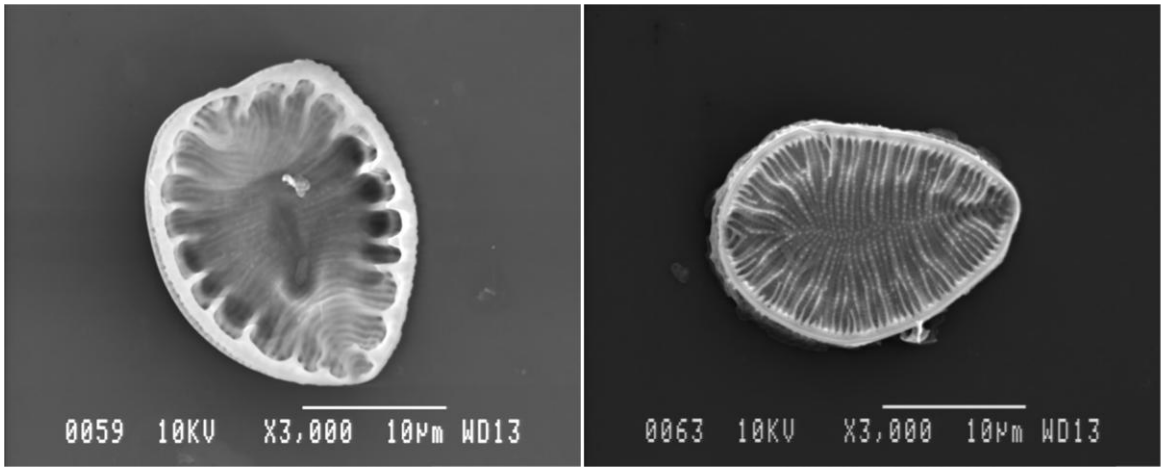


**Figure 5.1: SEM of *Surirella* on a glass cover-slip**

Then, for a better view, a drop of the acid rinsed sample is directly put on the stub and for quicker drying left it on a heating plate for 1 hr (temperature 23°C to 60°C). Screening the sample in the SEM

we observed that, by drying the sample on a heating plate, some layers covering the shell are also dissolved and remains are left which looks like a skeletal structure (figure 5.2).

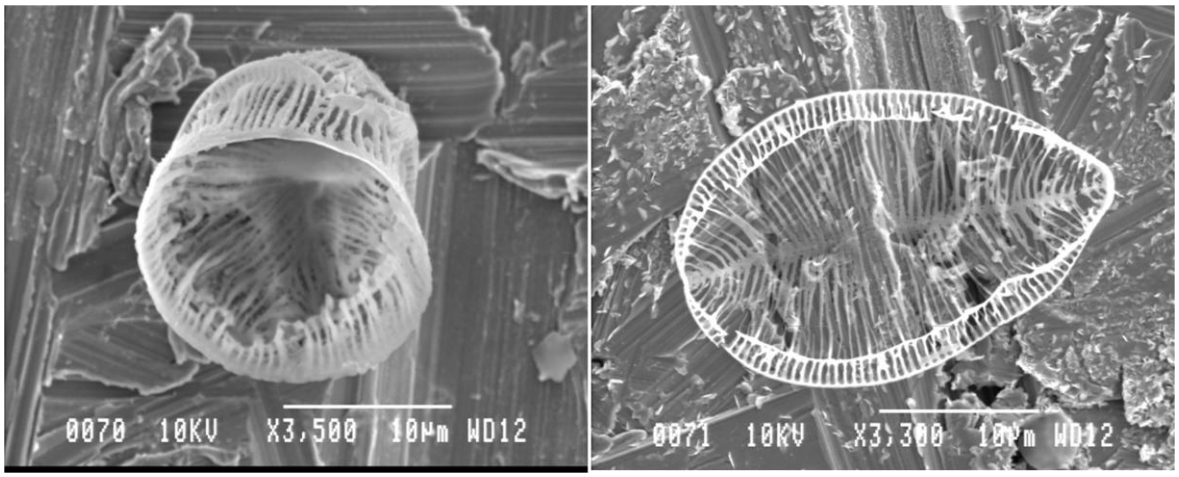


**Figure 5.2: SEM of *Surirella* diatom after drying on a heating plate**

The acid rinsed sample is filtered onto a thin filter paper, adhered to a stub; sputter coated with gold and also carbon coated using a JOEL's JEE-4X VACCUM EVAPORATOR since the fibrous surface deflects the beam and examined in the scanning electron microscope. Figure 5.3 shows how exactly the *Surirella* diatom looks like and a magnified view of the frustule structure. The perfect structure of the *Surirella* diatoms is obtained.

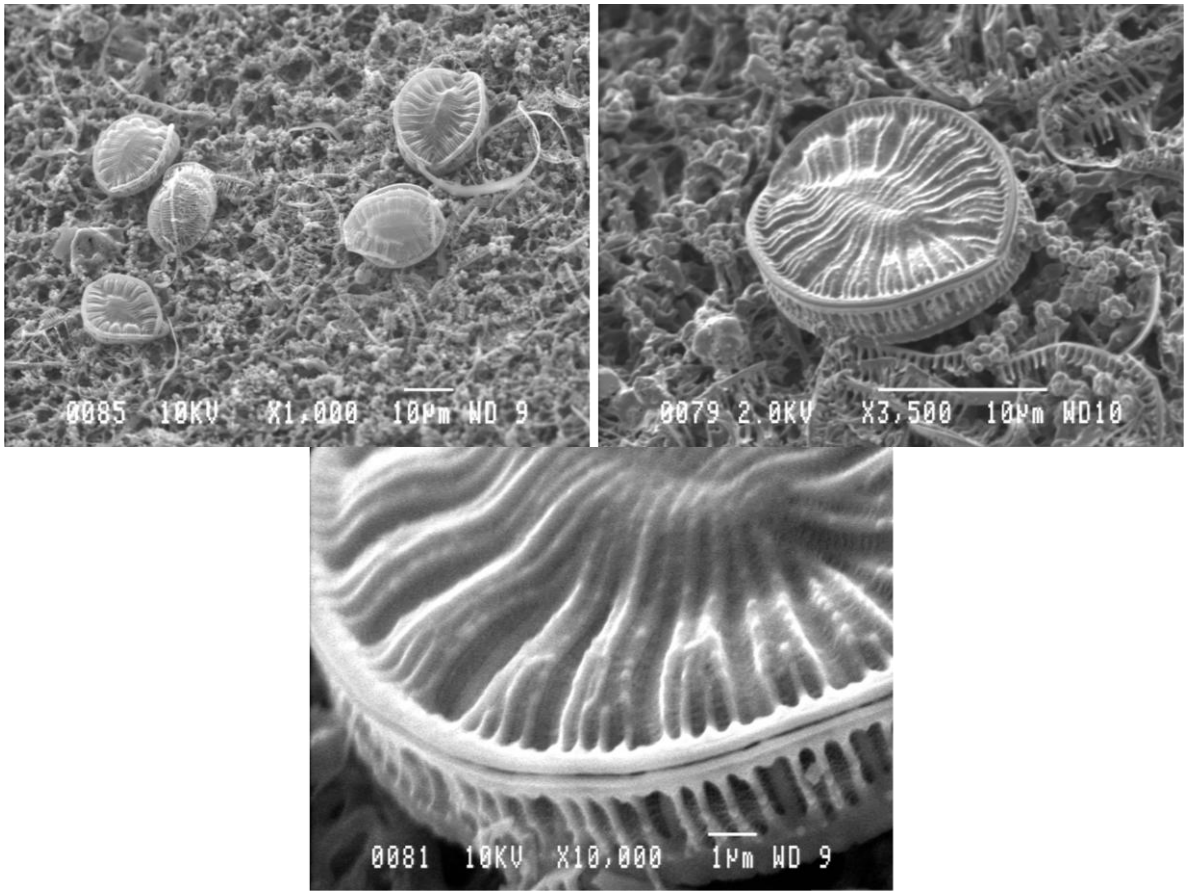


**Figure 5.3: Scanning electron microscopy of *Surirella***

## 5.2. INCORPORATING NUTRIENTS INTOA SILICONE ELASTOMER

For the orientation of the diatoms into ordered structure, we used a print technique, were organic or inorganic material is printed on to the glass plate. To make the pattern we use the PDMS stamps, fabricated as explained in section 4.1.2. The idea is to have a continuous slow release of nutrients from some elastomeric rubber kind of material, so that when this material is in the liquid medium a leakage occurs and the diatoms sense this and creep towards it.

One way of having an unhurried continuous discharge is to incorporate the nutrients into a silicone elastomer, which is easily available and also simple to transfer onto a surface. Different methods have been used to incorporate the nutrients into the silicone coatings, considering the miscibility of Sylgard with water and uniform distribution of particles.

One possibility could be directly blending Silicon Aerosil ($SiO_2$) (as a nutrient) in powder form with Sylgard. Or another is to mix the water solutions of the nutrients Si ($Na_2SiO_3 9H_2O$), P ($NaH_2PO_4 H_2O$) and N ($NaNO_3$) used in the culture media with the Sylgard by making emulsions, and then using them on the panels; and last but not least, directly mixing the liquid form of the nutrients with the Sylgard. The properties of the Sylgard used and the procedures followed for different methods and their corresponding results are discussed in the further sections.

### 5.2.1. SYLGARD® 184 SILICONE ELASTOMER

A porous silicone with open or interconnecting pores large enough to allow diffusion of water and nutrients through the silicone matrix is required. We have chosen Sylgard® 184, although it is not the most suitable carrier, however due to its biocompatibility and mechanical properties (i.e. elasticity, flexibility and tolerance to mechanical stretching) makes it best suitable for incorporating nutrients also suitable for considering the distribution of salt particle on the overall. The properties of Sylgard used are as in the Table II below.

| **COMPONENT** | ***SYLGARD® 184*** |
|---|---|
| Polymer base | **Vinyl-PDMS (90.9 %)** |
| Curing agent | **SiH-PDMS (9.1 %)** |
| Inorganic filler | **-** |
| Catalyst | **Pt (included in the curing agent)** |

**Table II: Properties of Sylgard 184 elastomer**

### 5.2.2. POROUS ELASTOMER USING AEROSIL ® OX 50

First and foremost we tried to incorporate dry form of Silicon into Sylgard since it is immiscible with water. Aerosil® OX 50 by Evonik Degussa Corporation is a power form of Silicon dioxide ($SiO_2$). It is a fumed oxide with the smallest specific surface area. High chemical purity, distinct low thickening and agglomeration properties are its typical characteristics along with being light in weight. Usually used in PET films, dental compositions or raw materials for production of ultra pure silica glass.

With its characteristic features, it was supposed that mixtures having higher concentrations could have been prepared by adding a large quantity of Aerosil® OX 50 to the Sylgard, but when tested, we have been limited to a range of 4-8% (w/w) of Aerosil in Sylgard. If increased above 8 wt%, then it becomes difficult to mix.

In our experiment 4% (w/w) Aerosil and Sylgard mixture (as it was a lot of Aerosil by quantity) is used. To make this mixture, 40 mg of Aerosil is added to 1 g of Sylgard and 0.1 g of curing agent (10:1 ratio) and mixed carefully, ready to use. Now we needed to verify the slow release of the Silicon Aerosil from the elastomer.

***Analysis for Aerosil Leakage***

Inductively coupled Plasma (ICP)[2] analysis is performed for detecting the amount of Silicon Aerosil leakage from the elastomer over time. For performing the analysis, 5 samples having a thin layer of the mixture with dimensions 1x4 cm were made on different glass slides. Each slide was put in a tube with 40 ml of MQ water at the same time. Then at particular intervals between 0-100 hrs, the slides were removed from the tubes and set aside. Finally all the samples are sent for analysis.

A normalized value is to be obtained since it was 5 samples each differing in the quantity of the Sylgard. In order to calculate this we needed the weight of the Sylgard layer. The glass slide is weighed with the layer, then the layer is scrapped off and again weighed; the difference in weights is the weight of the elastomer. The ppm value of each sample obtained from the ICP analysis is divided by the corresponding weight of Silicone elastomer layer, giving us the normalized value. These results of the analysis are presented in the graph below.

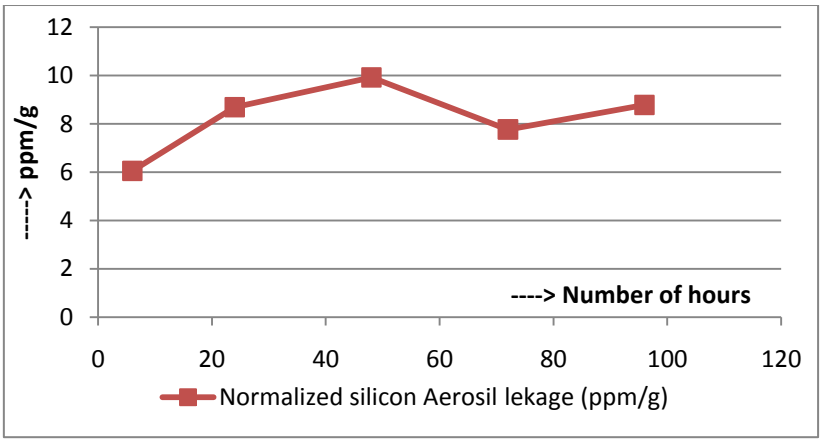


**Figure 5.4: ICP analysis of Silicon Aerosil leakage**

The release is good until 70 hrs, but after that there is a sudden drop. This could probably be because of the uneven distribution of the Aerosil in the mixture, and in the particular sample there could be a less number of Aerosil particles.

### 5.2.3. POROUS ELASTOMER USING LIQUID NUTRIENTS

In the previous method particle and dry form of the nutrient is used to produce a porous elastomer and as an uneven distribution of the Aerosil in the elastomer is noticed, as an alternative for manufacturing porous elastomer, using the diluted form of the nutrients has been tested. By using the water dissolved form of the nutrients Si ($Na_2SiO_3 9H_2O$), P ($NaH_2PO_4 H_2O$) and N ($NaNO_3$), there is expected to be uniform distribution and slow leaching of nutrients from the elastomer.

#### 5.2.3.1. LIQUID NUTRIENTS ALONG WITH EMULSIFIER

[2] ICP analysis is an optical emission spectroscopy for detecting trace metal elements by using inductively coupled plasma (argon, in our case), that produce excited atoms and ions which emit electromagnetic radiation at characteristic wavelengths of a particular element. Intensity of this emission is indication of the concentration of the element in the sample. Technique is highly sensitive, varies by element from 1ppm to 10ppb.

In order to make porous silicones using liquid porogens[3] (such as water), there is an intermediate step of preparing a water-in-silicone emulsion. This intermediate emulsion formation step is challenging, since such emulsion is usually unstable and water molecules may tend to accumulate as a separate layer.

By making an emulsion, the immiscibility of Sylgard with water is overcome as one liquid is dispersed in the other by forming a water-in-oil type emulsion. To enhance the stability of the water-in-silicone type emulsion, an emulsifier or surfactant is needed. Such emulsifier or surfactants reduce the surface tension of the liquid and make an emulsion more stable by lowering the interfacial tension between two liquids.

According to the properties of emulsions, to incorporate the liquid nutrients in to the elastomer, EnviroGem®360 (surfactant) and n-hexane EMPLURA® (solvent) were used. To 10 ml of Sylgard 1 ml curing agent, 0.5 ml of each of the liquid nutrients and 2.5 ml of n-hexane were added and mixed. The mixture turns form transparent to white in color. Then 0.5 ml of EnviroGem®360 is added and left in vacuum at 100 mbar pressure for 1 hr. This mixture was stable even after 24 hrs, showing no sign of a separation. A thin layer was spread out on a glass slide and cured for 1 hr at 100°C, resulting in not being cured.

It is probably the surfactant that contains Sulphur which poisons the Platinum in the catalyst, inhibiting the curing. Although more amount of the curing agent was added to the mixture, it did not do any alteration. This mixture does not cure even if it is left for days at room temperature.

#### 5.2.3.2. LIQUID NUTRIENTS WITHOUT EMULSIFIER

After failing to incorporate the nutrients into silicone elastomer by emulsions, we tried to mix the nutrient solutions with the Sylgard. Surprisingly, it was found that by mixing the liquid nutrients and the elastomer in normal environment or in vacuum, the need for emulsifiers can be avoided.

To test this method, first we tried to mix 10% (w/w) nutrient solutions and Sylgard. To 1 g of Sylgard, 0.1 g of each $SiO_2$, $PO_4$, $NO_3$ was added and also 0.1 g of curing agent, all these are mixed thoroughly but will little effort. As a result it mixed very evenly without any separation between the water and elastomer. Subsequently to confirm, a thin layer of the mixture was spread out on a glass surface and cured at 100°C for 1 hr, when it was taken out of the oven, the layer is transparent in color indicating that the layer is completely cured.

The nutrient solutions are liquids and it is not appropriate to measure them by weight. We tried to make 10% (v/w) nutrient solutions in Sylgard mixture. To make the mixture, 0.1 ml of each of $SiO_2$, $PO_4$, $NO_3$ are added to 1 g of Sylgard, and also 0.1 g of curing agent is added, all these together are difficult to mix. It takes time to mix up all the salt solution into the Sylgard then the mixture is set to settle for 30 min to confirm that it is a good emulsion without any separation. The concentrations of the salts in elastomer were as calculated in table III.

3 Sacrificial fillers (both solids and liquids) help in generating the pores in the elastomer during manufacturing of porous silicones.

| Component | Stock solution | Quantity | Weight of nutrients | Concentration in silicone elastomer |
|---|---|---|---|---|
| $NaNO_3$ | 75 g/L $dH_2O$ | 0.1 ml | 7.5 mg | 0.75 % |
| $NaH_2PO_4H_2O$ | 5 g/L $dH_2O$ | 0.1 ml | 0.5 mg | 0.50 % |
| $Na_2SiO_39H_2O$ | 30 g/L $dH_2O$ | 0.1 ml | 3 mg | 0.30 % |

**Table III: Concentration of nutrients in the elastomer**

A thin layer is spread out on a glass slide and cured at 100°C for 1 hr. No problem in curing is observed as a transparent layer is obtained; consequently making it possible for us to achieve 10% (v/w) nutrient solution in the elastomer.

***Analysis for Nutrient Leakage***

For analysis of the nutrient leakage over time, a similar procedure was followed as it was with Silicon Aerosil (described in the previous sections). The results from the analysis are as seen in the graph below which are also normalized by calculating the weights of the samples as described before.

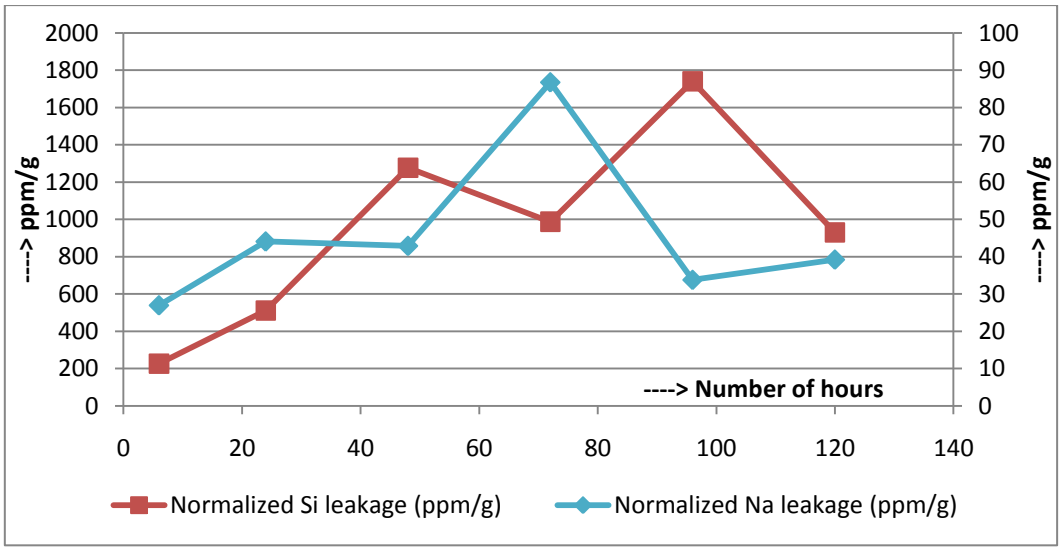


**Figure 5.5: ICP analysis of Nutrient leakage**

It was difficult to measure the presence of the other nutrients N and P, as the concentration was low. Thereby, we only have the measurements of Si and Na.

## 5.3. GROWTH OF *SURIRELLA* ON DIFFERENT SURFACES

The growth of *Surirella* was tested on four types of surfaces, as a subsequent step to successfully incorporating the nutrients into the silicone elastomer. Figure 5.6 shows a schematic of our experiment. The glass surfaces were coated by a layer of silicone elastomer incorporated with different salts, as described before. Subsequently, the glass surfaces that were modified with silicon Aerosil, nutrient solutions and Sylgard, along with an un-modified glass surface were placed in a polystyrene dish. Since the four samples were placed in one dish, we could assume that the culture conditions were identical for all.

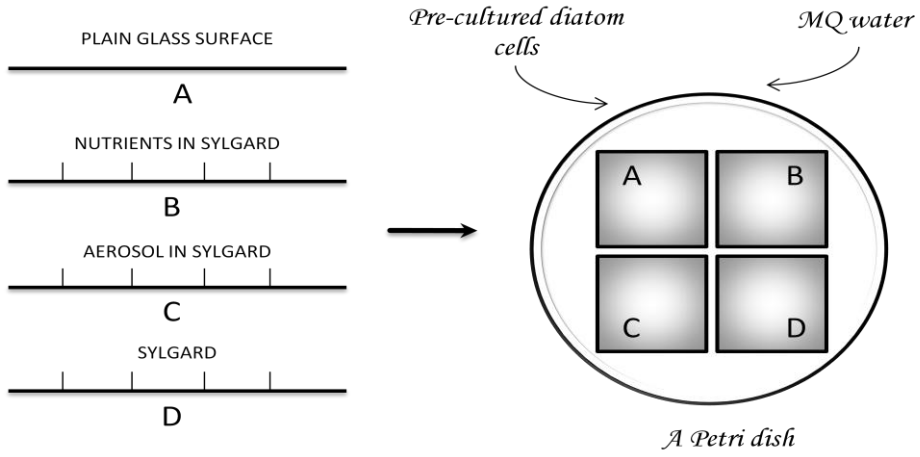


**Figure 5.6: Schematic representation of the experiments. Modified glass surfaces A, B, C and D were put in one Petri dish**

Subsequent to filling the dishes with 50 ml of MQ water, a pre-cultured *Surirella* suspension (10 ml) was dropped into the dish. The modified glass surfaces were completely submerged in the experiment medium and this setup was left for 2 days (day light and night light). Figure 5.7 shows the photograph of the above mentioned samples in one Petri dish that were cultured for 2 days.

**Figure 5.7: Photograph of diatoms cultured for 48 hrs on 4 different glass surfaces**

After 48 hrs, the samples were taken out of the medium and left to dry for 10 min, and then inspected under the optical microscope. Figure 5.8 shows the magnified images of the diatom arrays grown on the modified glass surfaces. In the images we could capture only a part of the surface, so to have a confirmed result we have calculated the average diatom count on all the four different surfaces (figure 5.9). The data clearly demonstrate that the number of cells on the nutrient modified surface is extremely high and also densely packed diatom cells were observed in this case.

To further see what happens to these diatoms when they are left for another 24 hrs without any culture medium, we have made an average count after 72 hrs also (in figure 5.9). From the graph it shows that as the modified surfaces have slow release, the diatoms do not die and seem to be increasing in number. Furthermore, this growth is higher on the surface with nutrients than others, but we cannot say if any of them are dead as it is a very large number and also difficult to observe. Nevertheless, it surely indicates that nutrient modified surface is superior to all.

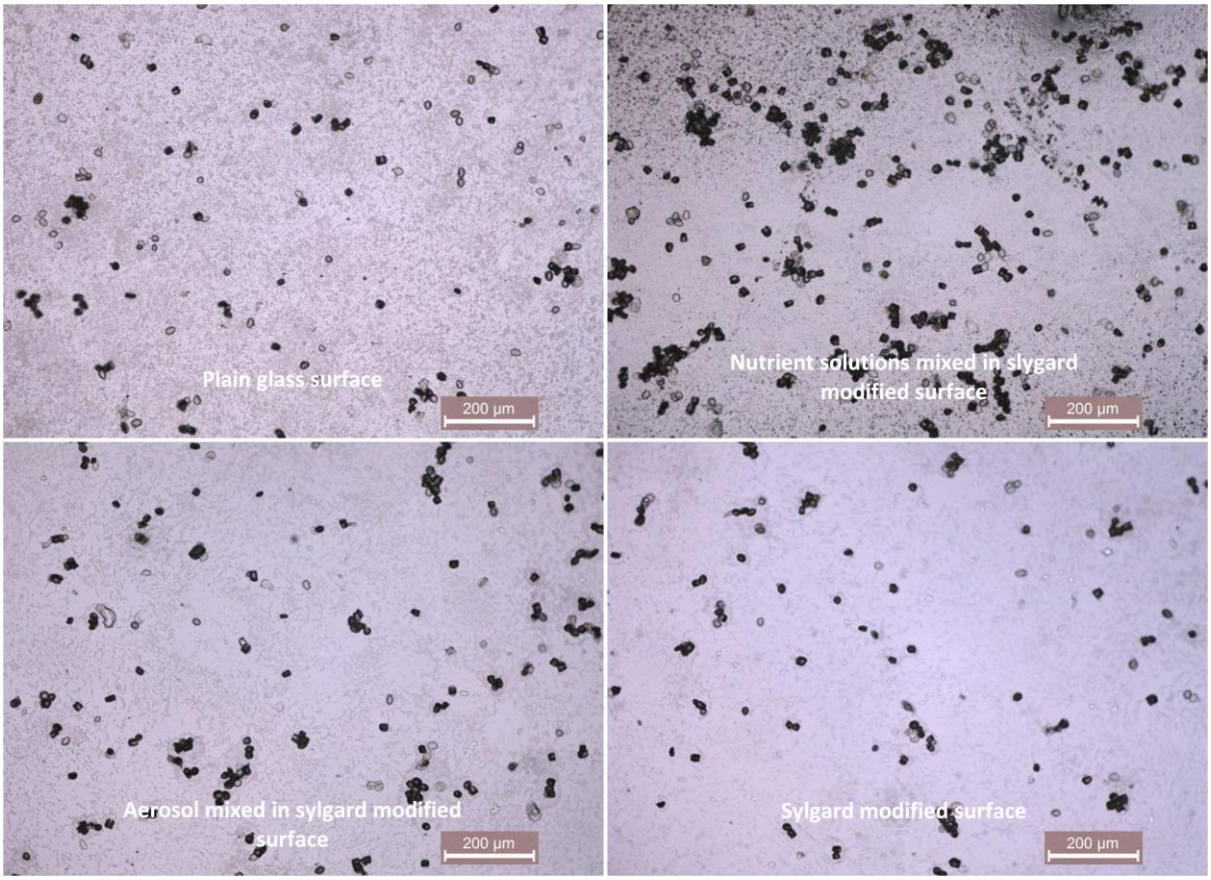


**Figure 5.8: Optical microscopy images showing the variations in the diatom number on different modified surfaces after 48 hrs**

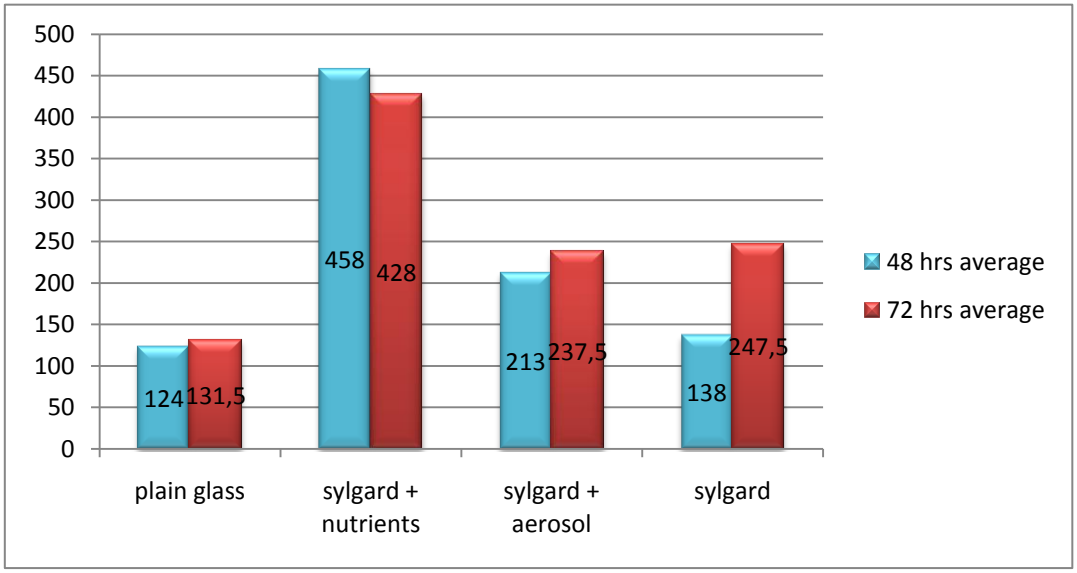


**Figure 5.9: Diatoms count on different modified surfaces after 48 hrs and 72 hrs**

From the above statistics it can be understood that, the surface with plain Sylgard and the surface with Aerosil in Sylgard did not differ much in the number of diatoms, so it could be that the Aerosil, being in particle form, does not have much influence in attracting the diatoms. Between plain glass surface and surface modified with nutrients in Sylgard a large difference is observed, so it can be anticipated that the diatoms could be oriented into a position by attracting with a coating of Sylgard

containing nutrients, which is the next step in the project. The results confirm that diatoms are more attracted to the surface modified with nutrients.

## 5.4. TRANSFER OF NUTRIENT LOADED SILICONE ELASTOMER ONTO GLASS SURFACE TO MAKE AN IMPRINT

The silicone elastomer is printed in a pattern by the PDMS stamp fabricated (as described in section 4.1.2), which has pillar like structures in an array.

For the transfer, first the Sylgard mixture having nutrients (procedure 5.2.3.2) is spread as a thin layer (~20 microns thick) on a glass plate by doctor blading method. Then a transfer is made with the stamp; a manual pick and place machine (figure 5.11) is used, for better accuracy and good imprint, to which the stamp is fixed.

### *Doctor-Blading the Sylgard Paste*

A portion of the paste is applied on top of the glass between the two pieces of Aluminium foil (approx 20 µm thick) (figure 5.10). With a rigid squeegee, such as microscope object glass or silicon wafer cut piece, the paste is spread across the plate with the support of the foil on both the sides. The gap between the foil is filled with a layer of Sylgard paste. In the figure below, the Sylgard paste is spread out, but due to transparency and also a very thin layer, it is not clearly visible. The process is repeated until we have a reasonably homogenous layer.

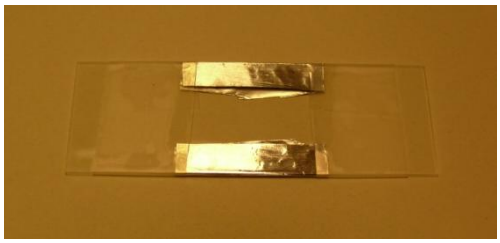

**Figure 5.10: Picture of doctor blading process**

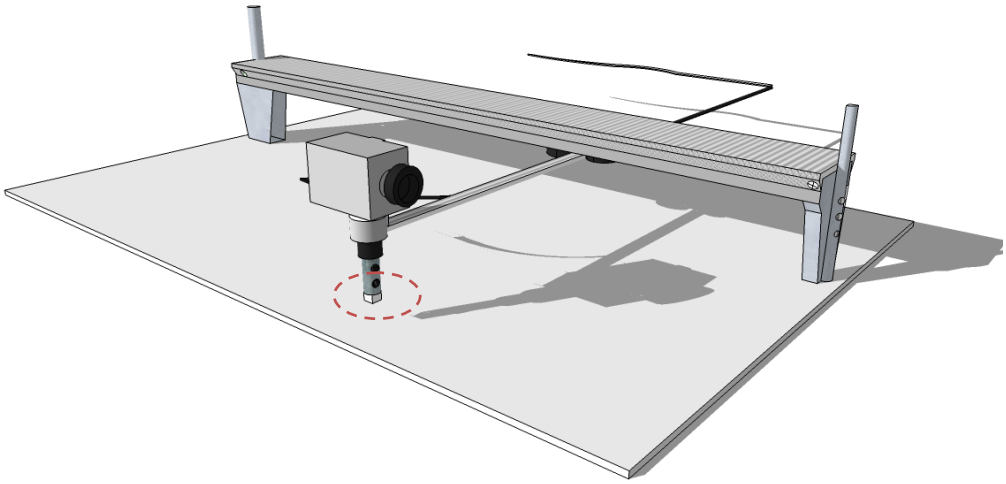

**Figure 5.11: Illustration of the Manual pick and place machine – for transferring the silicone elastomer**

Once the imprint is made on the glass slide, it is cured at 100°C for 1 hr. Inspected under an optical microscope; one of such imprints made is shown in figure 5.11. It was slightly tricky to make a homogeneous transfer; as few of the spots were bigger in size than the rest as we can see from the image below, but for the most part of the pattern looked quite the same. These imprints on glass are all set to be experimented.

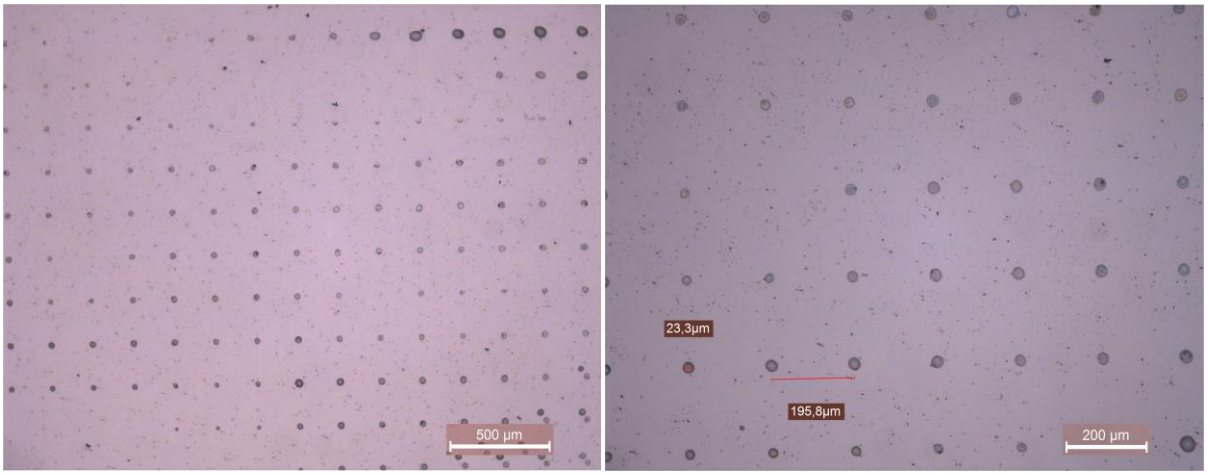


**Figure 5.11: Optical microscopy images showing transfer of elastomer**

## 5.5. RESULTS OBTAINED BY THE IMPRINT PROCESS - ASSEMBLY OF SURIRELLA ON SILICONE SPOTS

The glass slide with the pattern imprint is placed in a Petri dish containing 20 ml MQ water and to this 2ml of pre-cultured *Surirella* suspension is added. This setup is left unmoved for 48 hrs (2 days and nights – natural light). After 48 hrs most of the medium is removed from the dish with a pipette without doing much disturbance; only a thin layer is left, which is left to dry for 1 hr. Then the slide is examined in an optical microscope, figure 5.12 shows images of the pattern.

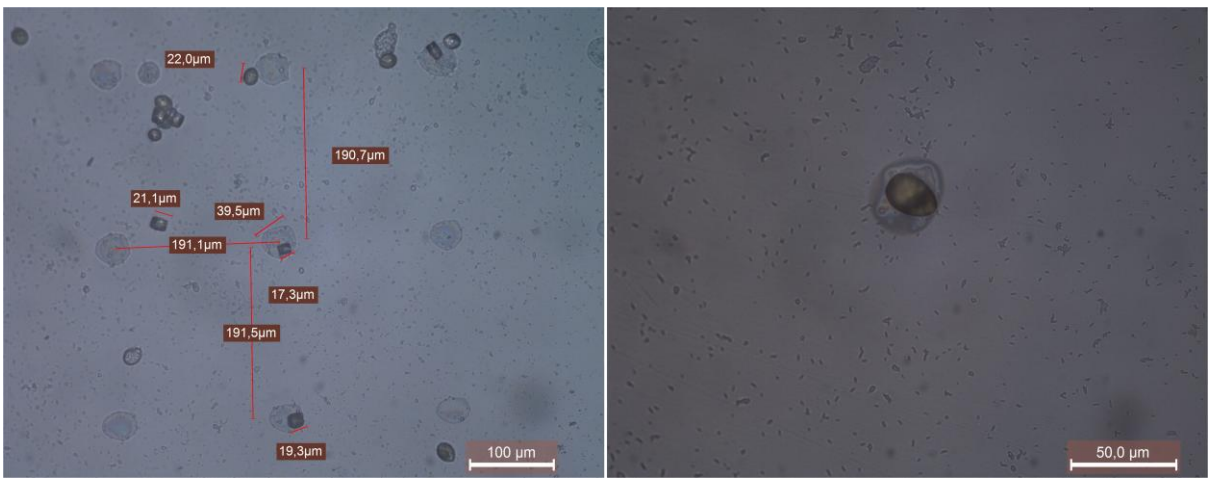


**Figure 5.12: Optical microscopy image showing the *Surirella* oriented on the silicone spots after 48 hrs (a) a part of the pattern (20x) (b) one diatom on one spot (50x)**

From the image we see that some diatoms are very near to the spots. Maybe if they are left for a little longer time, these would also have moved onto the spots, but it was not possible to test with these samples as they were already dried up, and it is not possible for the diatoms to glide without medium. Scanning electron microscopy was done to get a clearer view of the orientations after

sputter coating the samples with gold and carbon. Figure 5.13 shows the results, (a, b) imprints were made on a glass slide, as the chemical composition of glass and the spot seems to be the same, we cannot see a clear difference in contrast showing the difference between the spot and the substrate, but with a tilt of about 40°, these images show a difference in height, (c, d) imprint was made on a Petri dish, the contrast can be clearly observed. In figure 5.13 (b) we can see that the elastomer has developed a crack, which also indicates that the elastomer height is above the level of glass substrate and in figure 5.13 (d) the silicone elastomer spot is clearly seen, it is so bright because it has a higher atomic number than the substrate evidently indicating that it is Si.

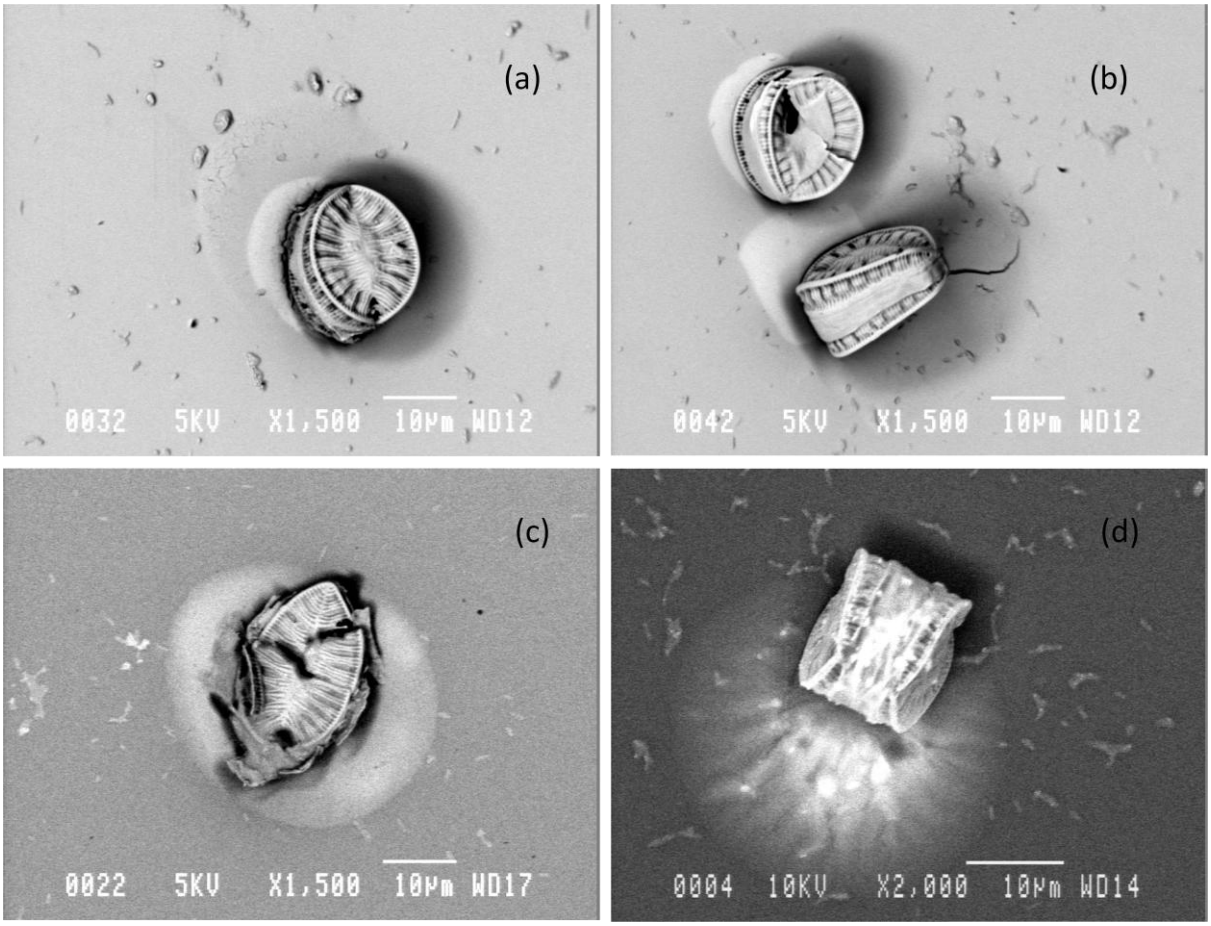


**Figure 5.13: Backscattered SEM images showing one diatom oriented on one spots; 2 different types of substrates (a, b) glass (c, d) Petri dish**

An X-ray analysis has been performed, which gives us the picture of distribution of different element on the overall. Figure 5.13 (d) is used for the analysis. The results of the analysis are in Appendix –II.

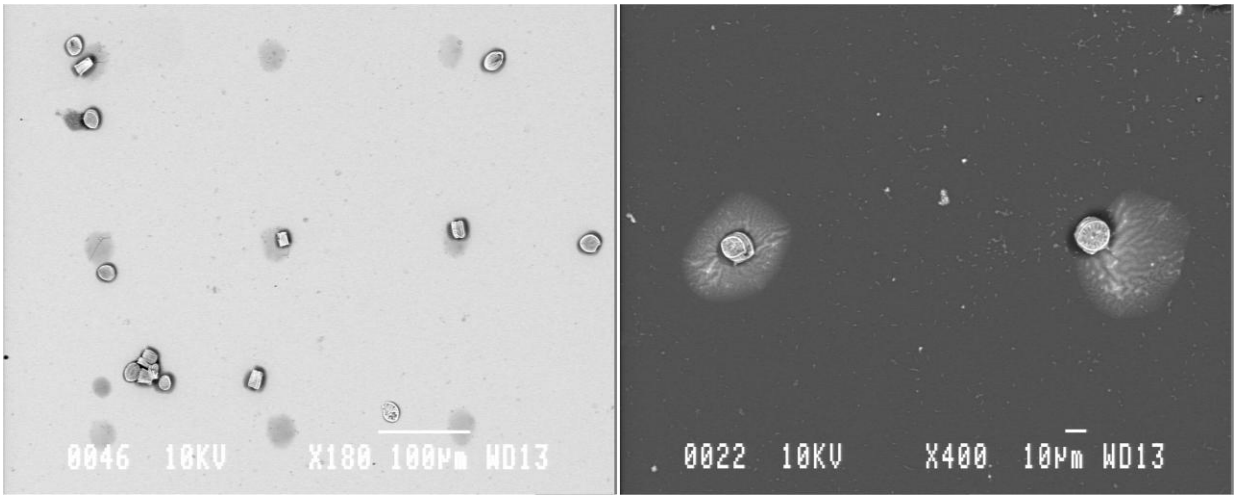


**Figure 5.14: Backscattered SEM images showing small areas of the patterns being oriented**

## 5.6. LIGHT THROUGH TITANIUM-ON-GLASS MASKS TO ORIENT *SURIRELLA* INTO A PATTERN

Apart from using nutrients in elastomer to orient the diatoms, we have also tried to orient them by just using light. As the diatoms *Surirella* are photosynthetic in character, they should be attracted to light, which is tested. For these tests, we use the titanium-on-glass masks (described in section 4.2).

To see if they are attracted to light or not, they should be in a dark environment and only limited light be given to them. For such an atmosphere, we made a setup as in figure 5.15, where a Petri dish is entirely covered in black from the outside, leaving just a small hole through which light can pass. The mask with pattern of 20 µm wide holes is glued to the dish with Sylgard (as it is transparent and allows light to pass through it). The setup is set in such a way that, the light generated by the light source falls on the mirror, on top of which the Petri dish with the mask is placed. This setup it in order to have control of the light intensity with flexibility to increase or decrease the intensity; and the mask looks as in figure 5.16, with and without illumination.

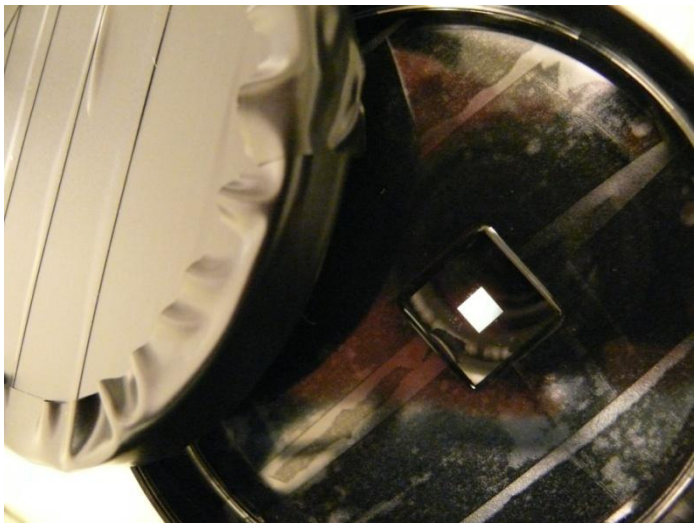

**Figure 5.15: Picture of the light experiment setup**

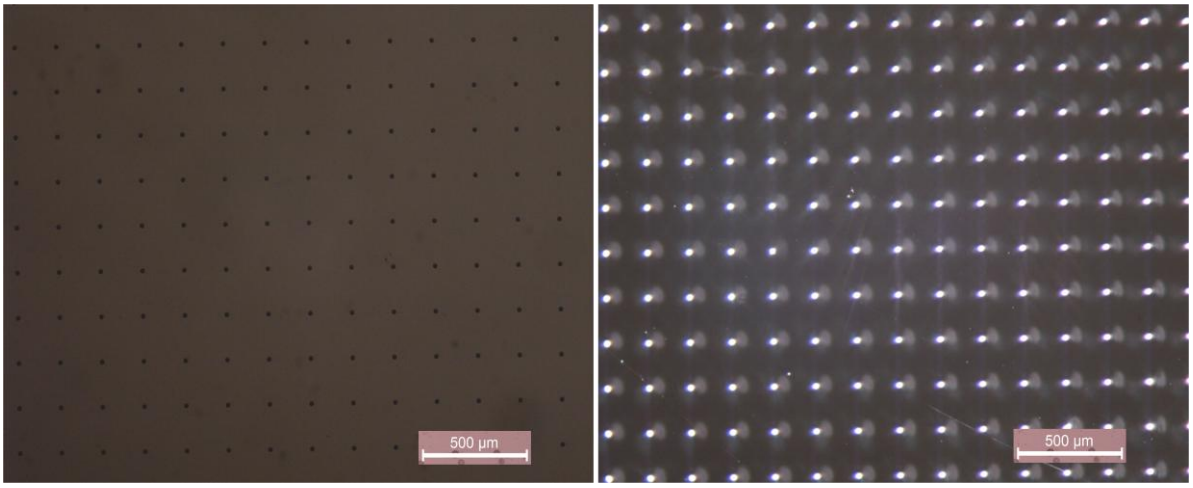


**Figure 5.16: Titanium-on-glass mass with 20 µm hole pattern without illumination (left) and with illumination (right)**

## 5.7. RESULTS OBTAINED BY THE LIGHT PROCESS

In the setup made for the test of light, the Petri dish is filled with 20 ml of MQ water and then 2 ml of pre-cultured *Surirella* suspension is added. After 72 hrs, nearly the entire liquid medium is removed by a pipette without doing much disturbance, leaving the rest to evaporate. Then it is viewed under an optical microscope. It works, we see many Surirella near the light spots and some also sitting on top of the light spots (figure 5.17). The intensity of light used is measured to be 150 µW in this case.

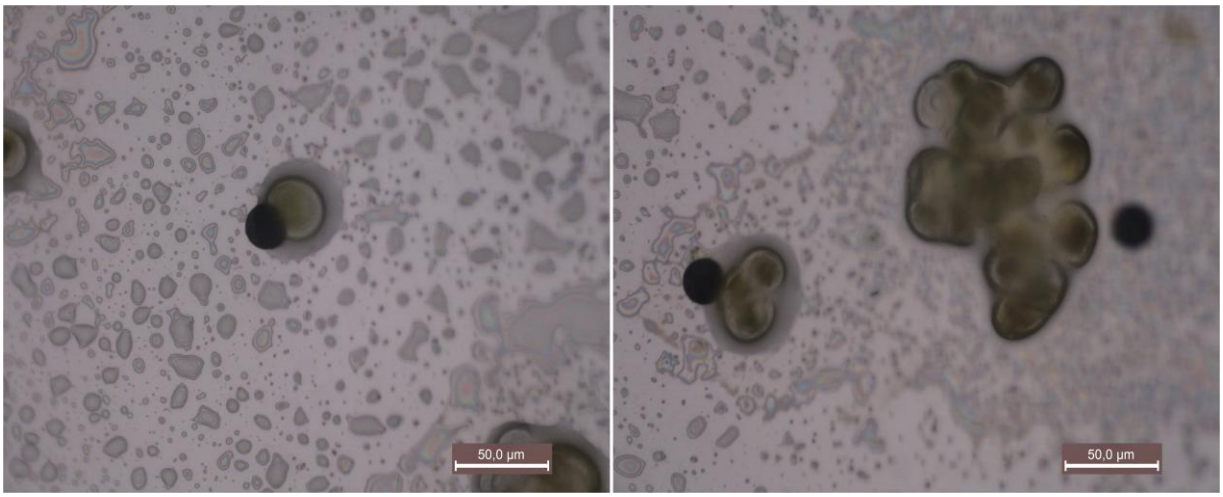


**Figure 5.17: Optical microscopy images showing *Surirella* positioned on the holes in the mask (dark round circles)**

The sample was tested with light at 150 µW, was left to dry completely for 2 days, then sputter coated with gold and carbon for SEM analysis, figure 5.18 shows the results.

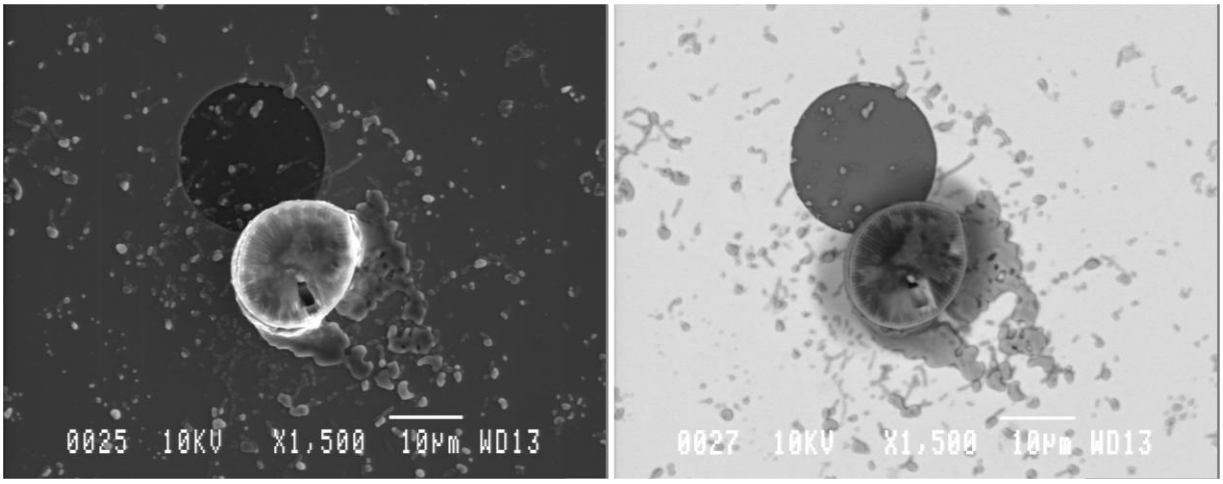


**Figure 5.18: SEM images of diatom sitting on the light hole in the mask; Secondary electron (left) and backscattered (right)**

In the whole pattern in the mask, only around a few holes, the diatoms had clustered, and there were many places, where the diatoms very near the holes, but as we had dried it after 72 hours, they are stagnant in that position and cannot move any further. Thus probably if the same sample was left undisturbed, we could have seen more of them being oriented. In Appendix - III are SEM images of the holes on which the diatoms are seen in big clusters.

## 6. DISCUSSIONS

The various results obtained and techniques experimented with have been discussed in the previous sections of this report. Here is a brief discussion about a few of the techniques used and also a discussion on a general level which could be implemented to obtain better results.

***Growth of diatoms on Modified Surfaces*.** In the past regulating the growth of diatom cells on chemically modified glass surfaces by using a technique of self-assembled monolayer's (SAM) has been tested by Kazuo; et al.[58] The growth of diatoms on different modified surfaces has been proved in our experiments where we followed a procedure similar to them, and we see from the results that the *Surirella* are attracted to a particular surface which has been modified with nutrients.

In a previous work: Self – assembly of nano-structured diatom micro-shells into patterned arrays, assisted by polyelectrolyte multilayer deposition and inkjet printing technique, diatom are arranged into rectangular arrays. This is a chemical method – where diatoms are dead by the time they are arranged. In comparison we work with living, hale and healthy diatoms in our project to form patterned arrays, which is a totally biological method.

There are only a very few topics where a discussion is possible, as this is a very new area of research. We experiment with a very new method, which could prove to be a foundation for an upcoming technique in the manufacturing field. There is a lot of scope in the future and we have proven that it is possible to orient the diatoms into a particular position, though not one hundred percent, as it is not possible for mankind to control nature completely.

*Chapter 7*

## 7. CONCLUSIONS

The synergistic combination of the biological assembly with synthetic orientation principles opens the doors to a large number of 3-dimensional micro/nano structures, which will have properties that can be tailored for a definite device application.

A method has been established which can be used for the orientation of the diatoms into patterned arrays. Orientation can be achieved by using both light and imprint of nutrients, for both the methods an experimental procedure can be accessed from the current project work which has been the main aim.

Several methods have been tested, preserving the life of the species and not using any chemicals. Light and nutrients have worked to orient the diatom microcells into positions. Silicone elastomer has been used for incorporation and slow release of nutrients over time. These approaches may be used to orient any kind of species among the 100 000 available according to the requirements.

Thus the main goal of the project to find methods to orient the diatoms is reached. With further alterations to these methods, much better results can be obtained as we now know a procedure that could be used for orientation.

*Chapter 8*

## 8. FUTURE WORK

Diatoms for Nano-manufacturing is a totally new area of research, which has been Per Johander's (supervisor) idea for the last 10 or more years approximately, and came into practical work just last year, 2010. Therefore, there is a lot of further work that can be done in this specific project itself and also on the whole.

From the establishing of better image acquisition systems to experimental apparatus, we could bring in very efficient tools and obtain better result. The next step in the path of orientation of diatoms could be ready after there is a following progress in the genomic part of the Vinnova project.

Another area of interest could be pair formation, where cues can be used to alter the behaviour of the *Surirella*. For this we need to know the signal substance responsible for the clustering or colony formation in them; this could be used in place of the nutrients to orient the *Surirella* into patterned arrays in a more efficient manner. Thus a bond will be formed between both the diatoms and this can be made permanent by sintering which will also immobilize them.

Future advances in genetic engineering of bio-mineralization in micro-organism, can lead to precise control over the morphologies of the frustule structures. Then this can be coupled along with the orientation principles. Furthermore, a change in the species could be thought about in the future, to see if a more active and fast moving diatom can be selected which will add to the experimental procedure and make orientation easier.

Thus, the combination of both genetic tailoring and pattern orientation could promise to make manufacturing of low-cost 3 dimensional micro/nano devices a reality.

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**APPENDIX – I**

***A.*** ***master.dwg (scheme):*** is the CAD design for the silicon master fabrication which was used for replication in PDMS.

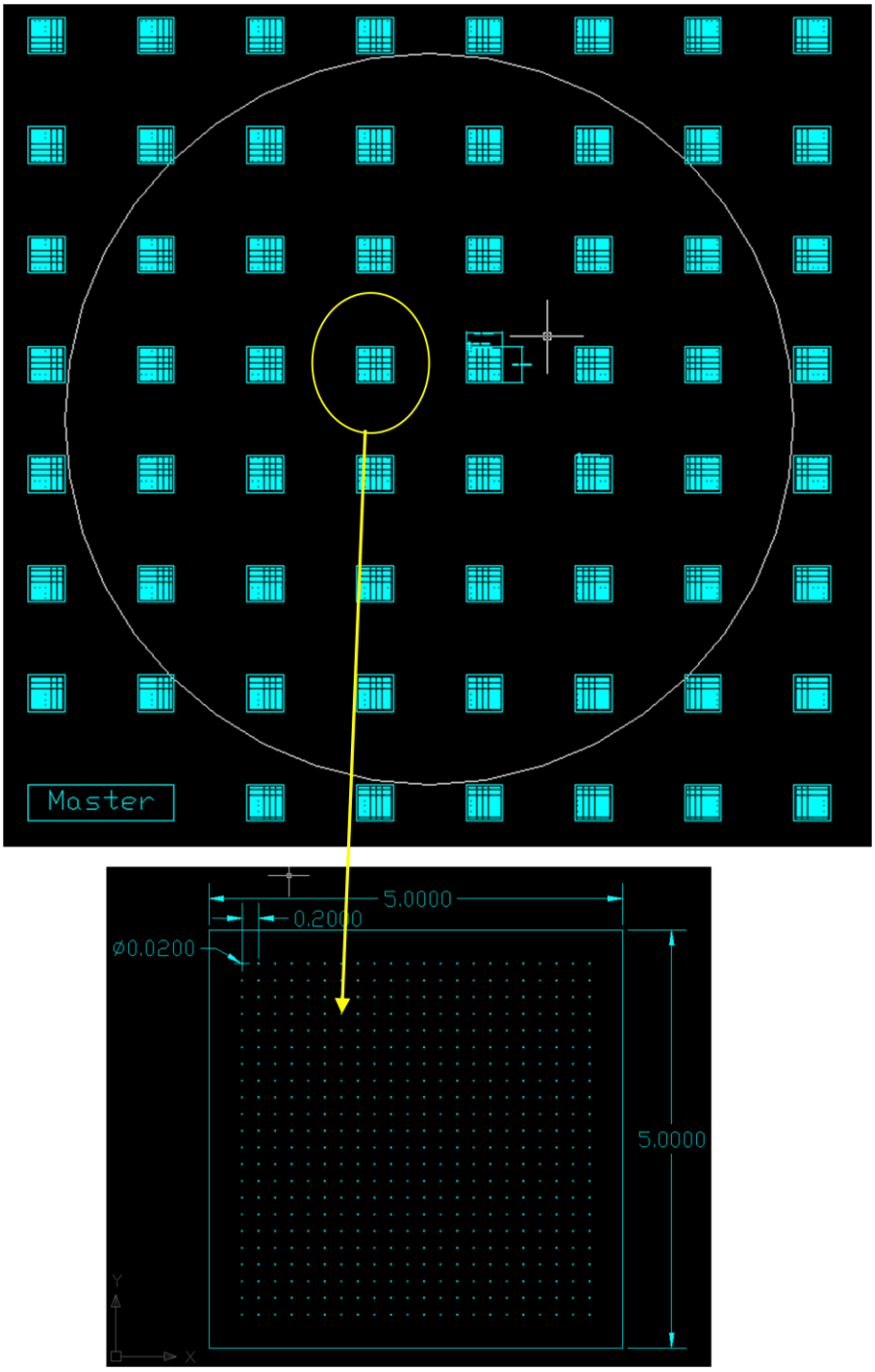



**Figure: CAD design for Silicon masters**

***B.*** ***single_double.dwg (scheme):*** the CAD design for the mask fabrication of titanium-on-glass plates.

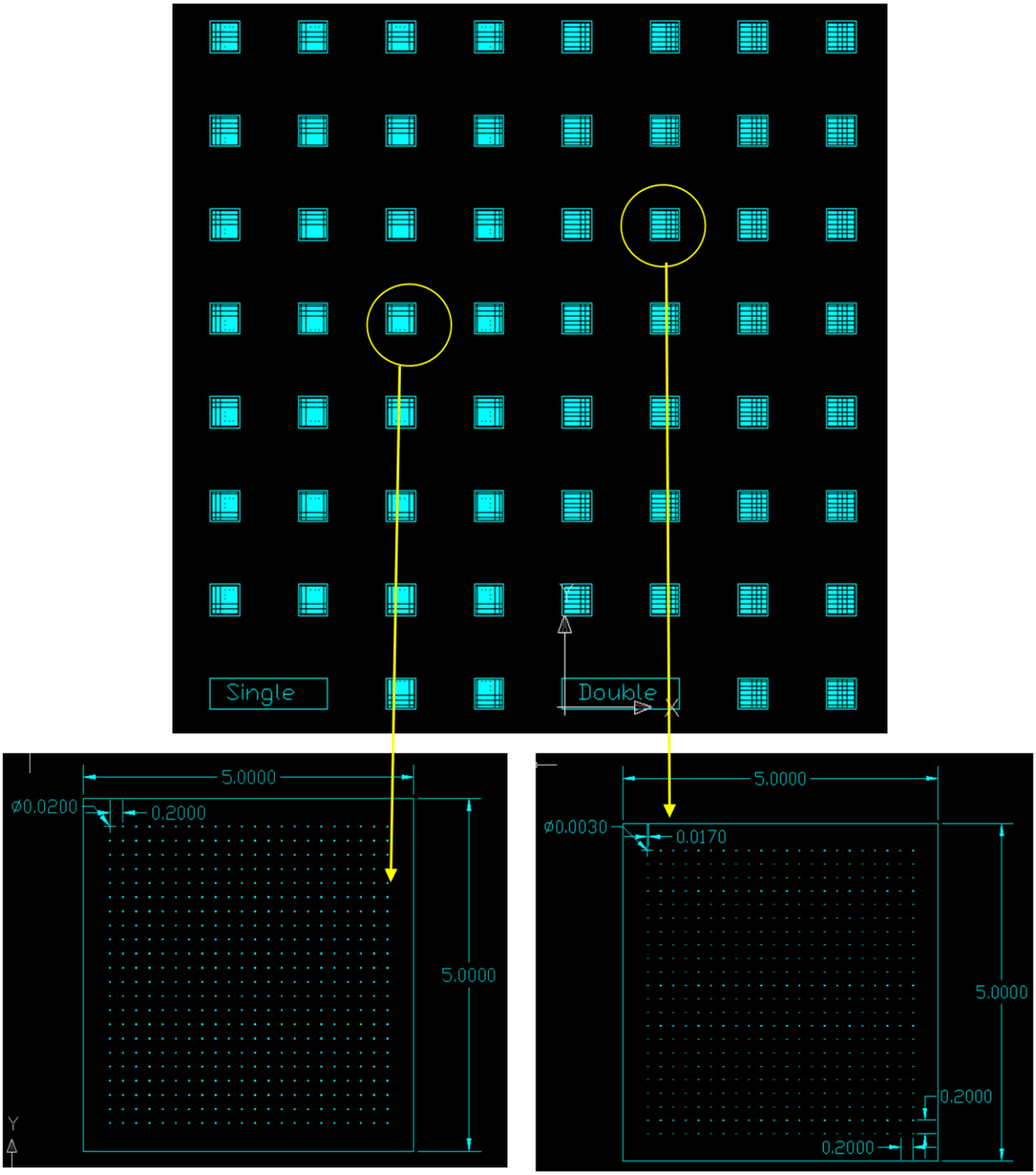
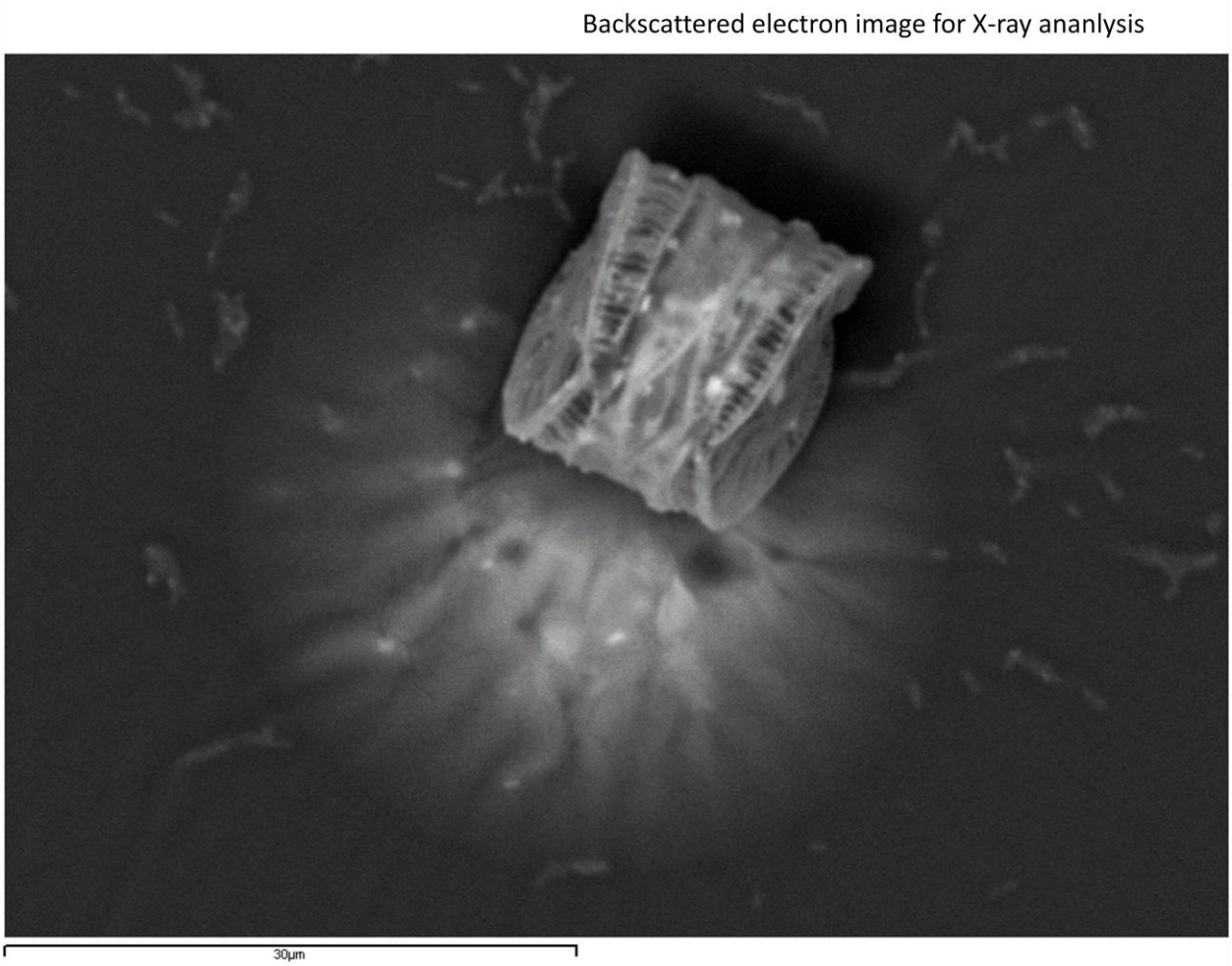
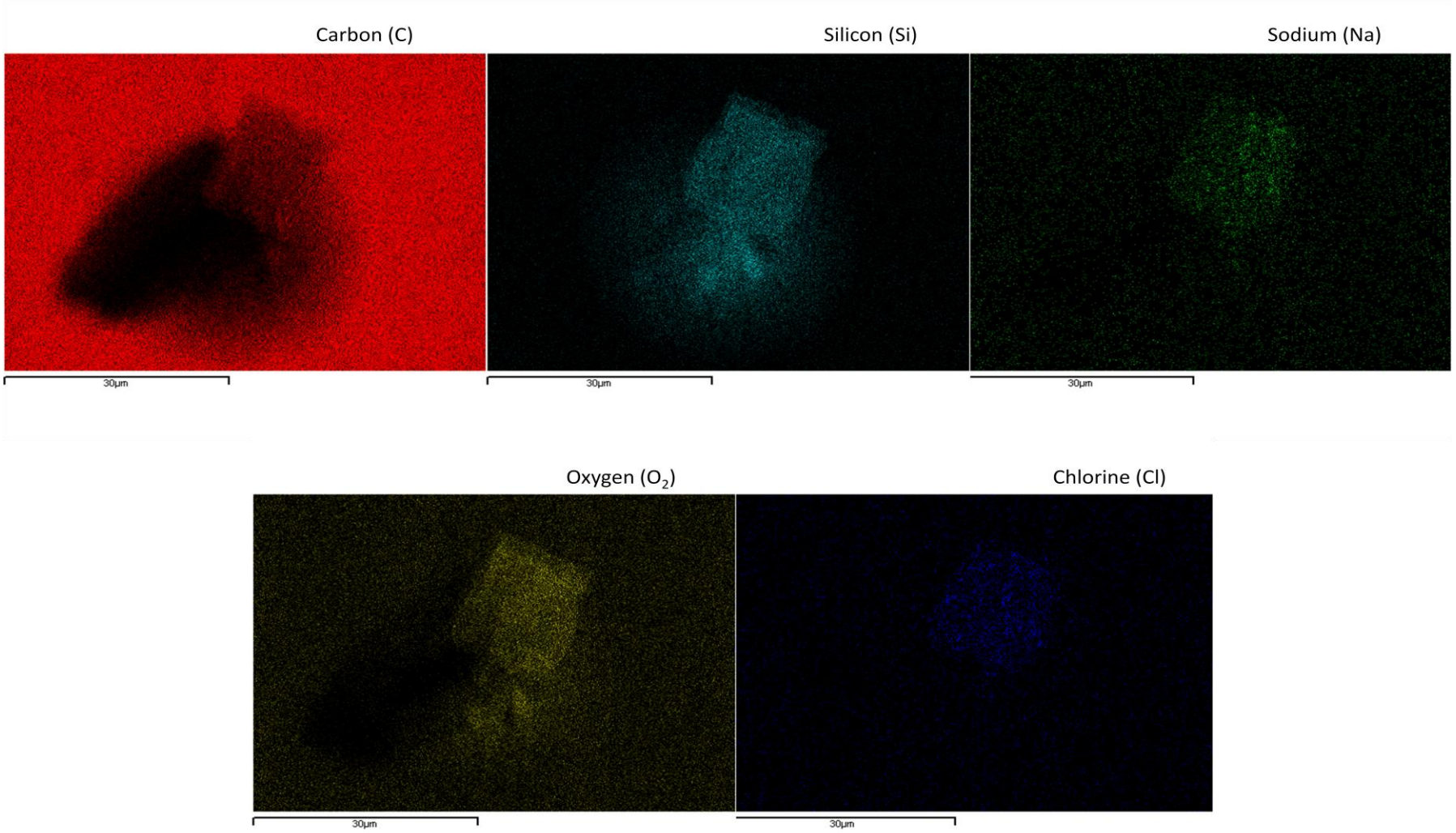



**Figure: CAD design for Titanium-on-glass masks**

**APPENDIX – II**

Results of the X-Ray Analysis for Silicon distribution

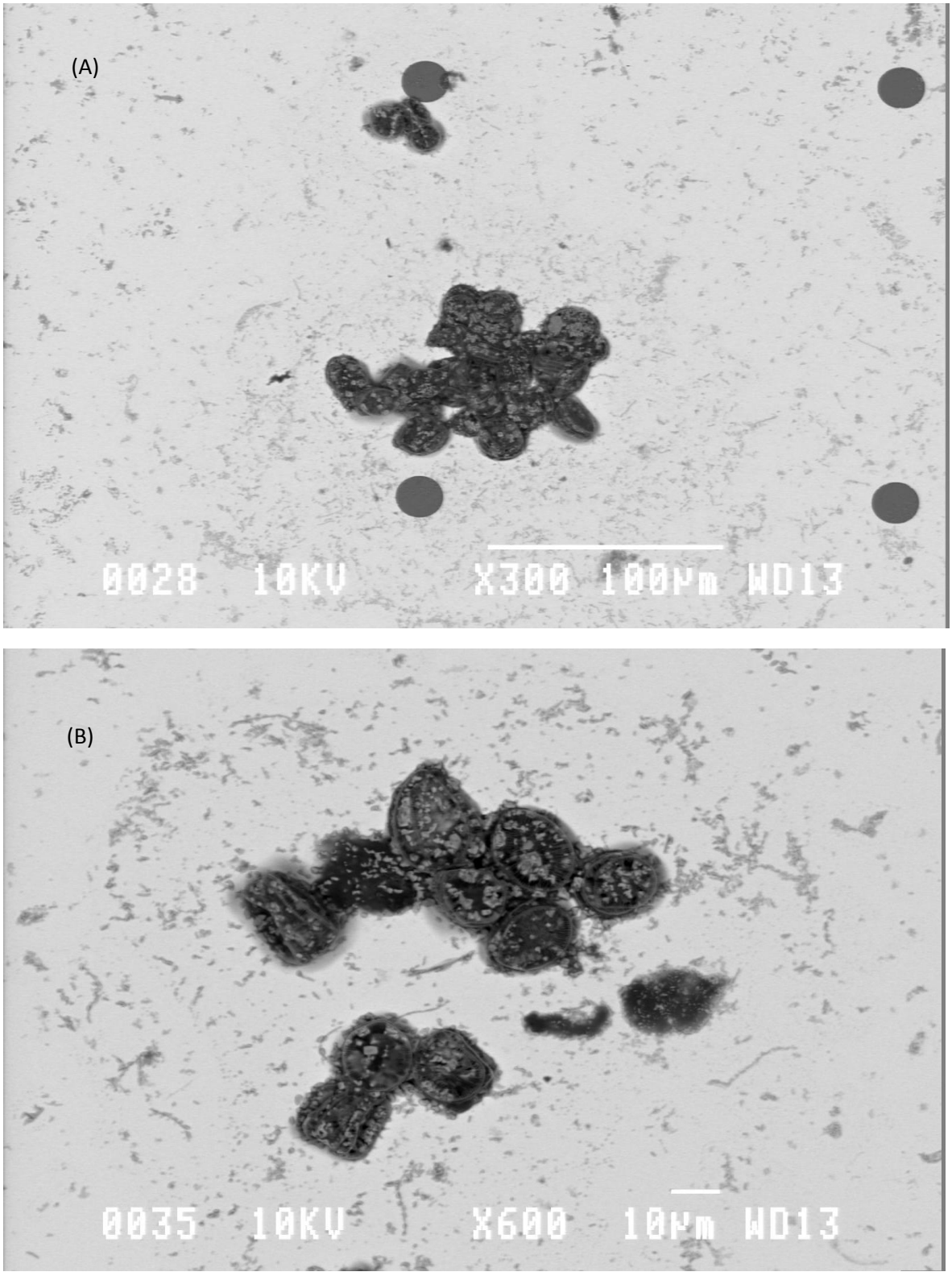




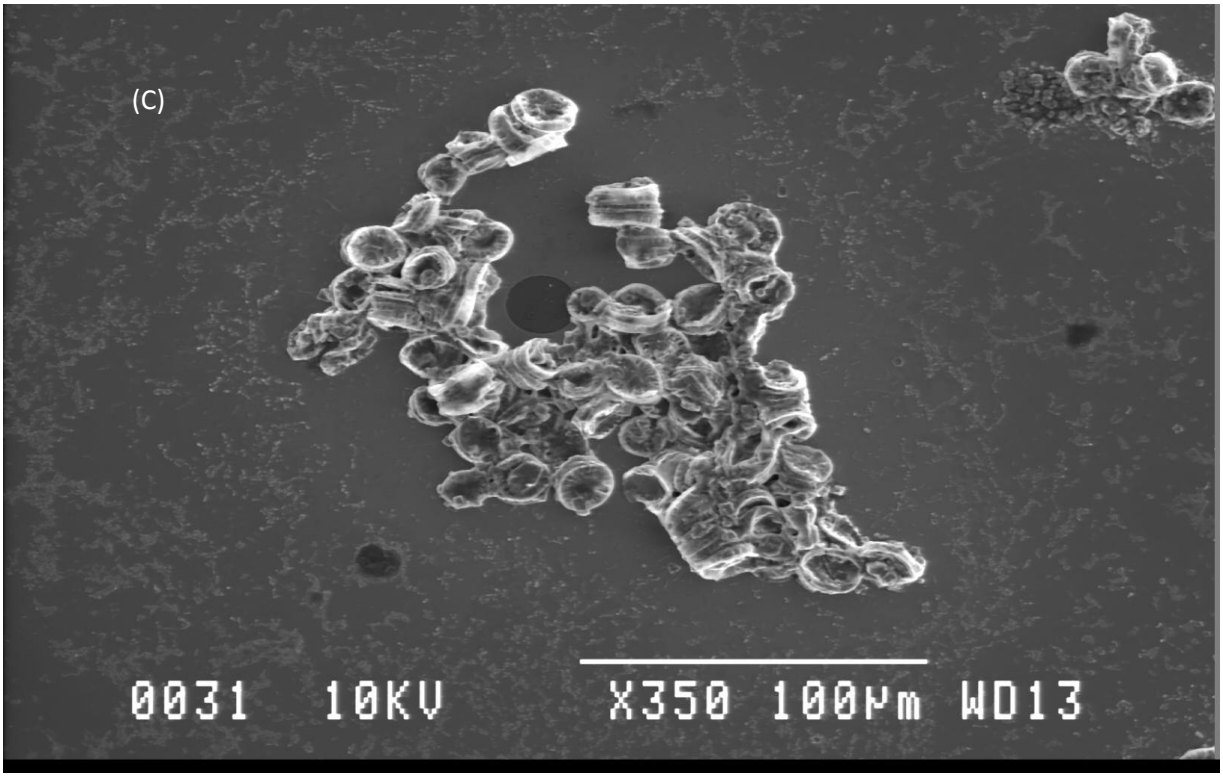

**X-ray ananlysis showing distribution of detected elements: *C, Si, Na, $O_2$, and Cl***

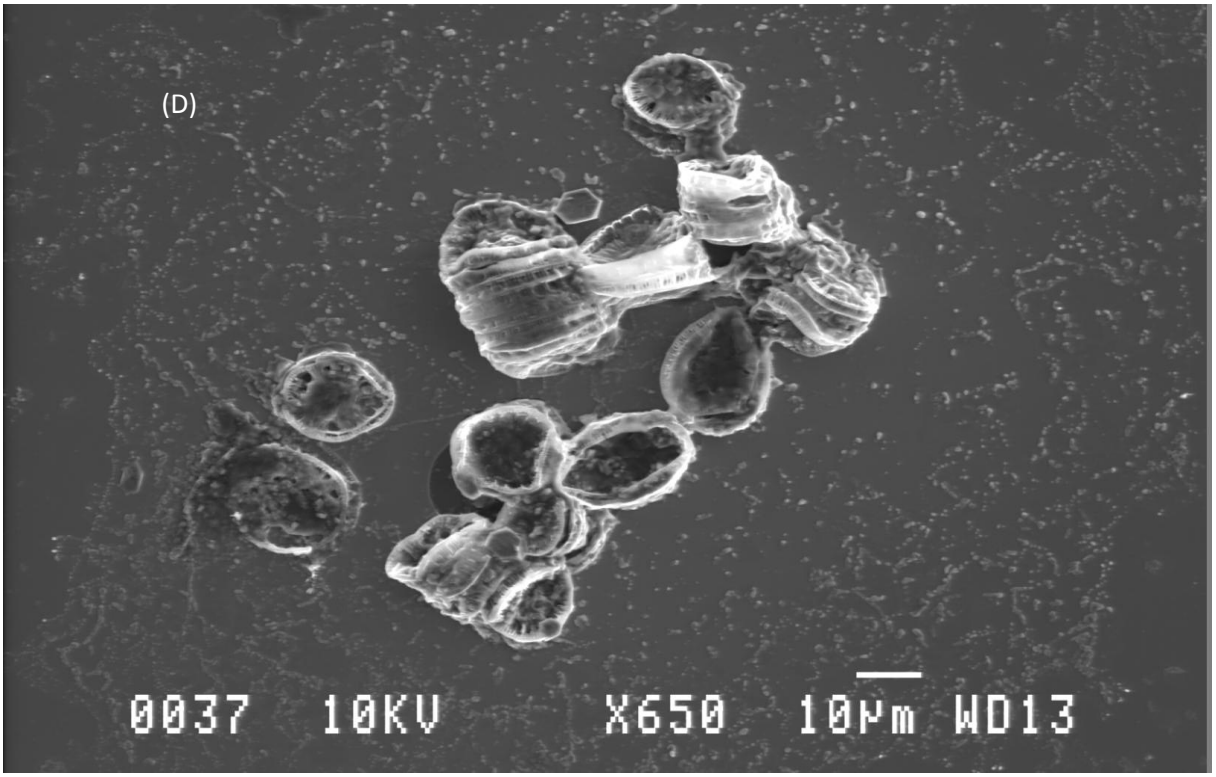
**APPENDIX - III**

SEM images of the results obatined by light experiements.




**A,B : Backscattered SEM image of *Surirella* diatom oriented on the holes in the titanium-on-glass mask**




**C,D: SEM images of few more holes with secondary electron beam**