University of South-Eastern Norway
Faculty of Technology, Natural Sciences and Maritime Sciences

Master's Thesis

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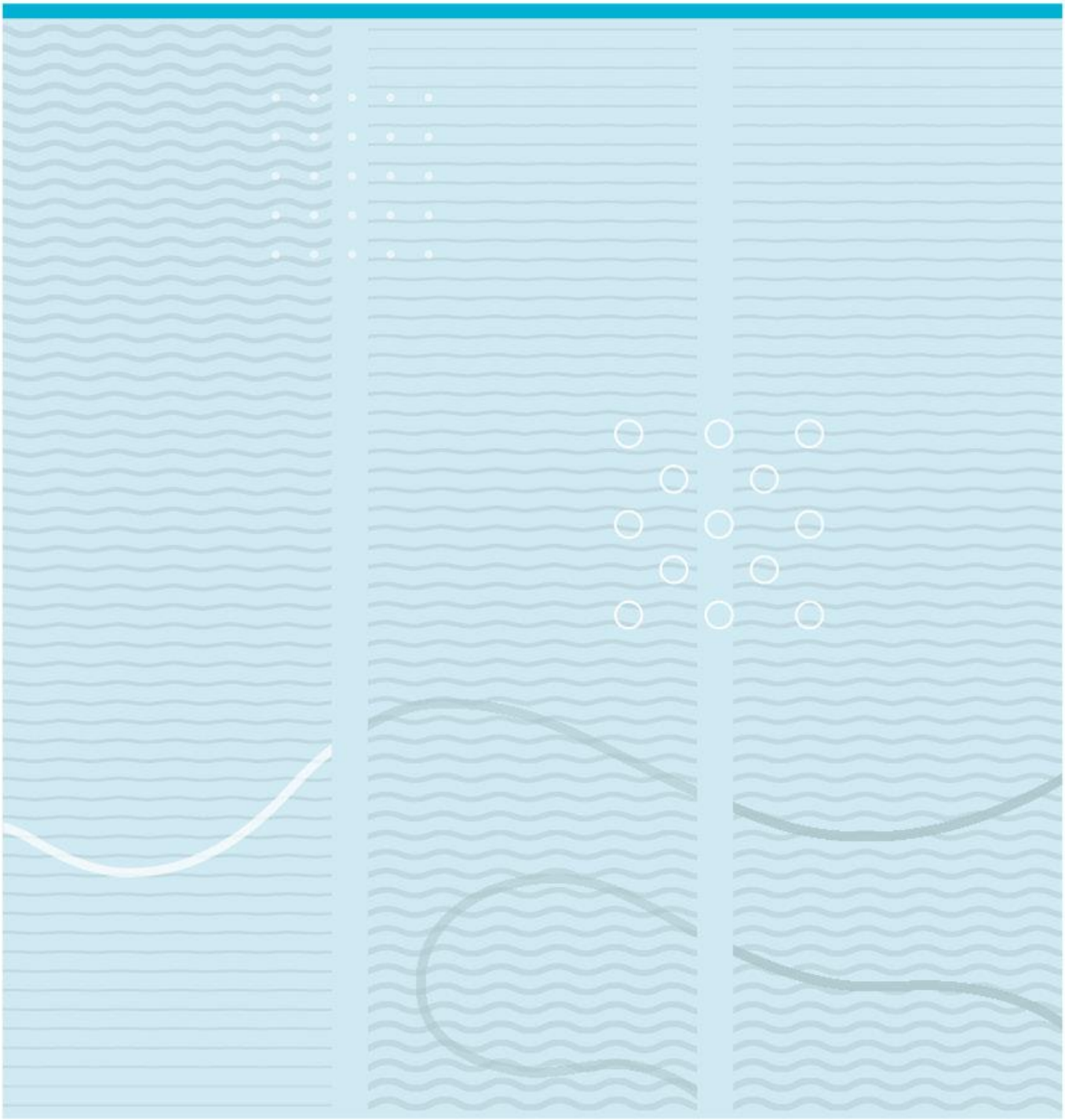
# Fabrication of clogging-free microfluidic chip for continuous separation of microparticles



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# Summary

The University of South-Eastern Norway has the plan to integrate a Centre for autonomous Particle Separation, Concentration and Refining (PASECOR) inside the new USN Innovation centre. Through research and innovation, PASECOR will deliver both a research based and sustainable technologies for purifying raw components from complex mixtures and solutions. The main goal of the proposed project was to further develop the PASECOR technology to make it ideal for separation and concentration of bacteria (1.0-0.2 micrometer) and microalgae (2.0-10.0 micrometer) from ocean water, lake water and raw oil solutions. From old versions of the PASECOR technology prototypes new fabrication methods and improved prototypes were designed and developed. These improved prototype include structures in order to improve the continuous separation, concentration and sorting efficiency, improve the flow through rate, improve the possibility to treat down to 5 micrometre particles and reduce the pressure drop to a minimum.

The new design consists of three chips including a top and bottom layer in borofloat33 glass and middle layer in silicon. The structured glass wafer was designed and evaluated in this project before fabrication in Germany. The silicon wafer is fabricated in IMS (Institute of microsystem) clean room by using micro and nano fabrication method, during this project. These three chips were supposed to be packaged together but the stack of three wafers with structure offered some potential challenges and work is still under progress

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# Preface

This thesis is the result of my master project at the Institute of Micro and Nano Systems Technology under the Faculty of Natural Sciences and Maritime Science (TNM). My supervisor in the group was Professor Frank Karlsen (PASECOR director) and my work in this group was part of a larger research project in refining field. During the fall 2019 I had to make a choice of what my master project was going to be about. There were a lot of different projects to choose from, but this project was stand out due to its unique concept and involvement of laboratory work. This project gave me a valuable insight into a fast-growing field within micro and nano technology and was directly helpful in my future assignments. Finally, I am heartfully thankful to Prof. Frank who gave me chance to work with him and learned from his knowledge and experience. I am also grateful to co-supervisor Prof. Lars and lab engineers to provide me support and assistance throughout the project

Borre /18/07/2020

ASIM NOOR

## Declaration of personal contribution

- Novel concept of the counter-flow unit:  Prof. Frank Karlsen
- Design of chips and masks: Asst. Prof.Lars Eric Roseng, Nhut Tran-Minh, Asim Noor
- Microfabrication: Asim Noor
- Assembly and bonding: Asim Noor, Asst.Prof. Lars Roseng, Prof. Frank

# Contents

# Introduction

## 1.1 Filtration

Since the ancient times, communities have employed techniques to separate and purify chemical substances for improving the quality of life. The extraction of medicines from the plants and metals from ores is older than documented history. Currently, separation process is crucial for many vital industries; the petroleum industry splits crude oil into useful products utilized as fuels, lubricants and chemical raw materials, [1], the pharmaceutical industry separates and purifies natural and synthetic drugs to cure diseases [2]; the mining industry is based on separation and purification of metals and the main challenge in inventing the atomic bomb was the separation of uranium-235 from uranium-238 [3].

There are mainly two reasons for performing filtrations on mixtures. First the mixture may contain some contaminants that should be removed from the rest of the mixture: this process of isolating and thus displacing is called purification like separation of microorganisms from water. The second reason is to transform the composition of a sample such that one or more of the components can be inspected, i.e. the investigation of air pollutants to assess the air quality [4].

The need for filters grew with the advancements within biological and medical fields. Following is the history describing developments of filtration process through ages.

## 1.2 Evolution of a Filtration Process

### 1.2.1 Greece 2000 B.C.

Water treatment documentations register back over 3500 years when civilizations used to boil and strain water to eliminate undesired substances. Around 500B.C. the first known domestic filter emerged when the Greek scientist Hippocrates fashioned the Hippocratic sleeve which was composed of a simple cloth back filter.[5]

### 1.2.2 The Egyptians (400 A.D.)

Ancients sketches and paintings from the Egyptian tomb clearly provides the evidence that Egyptians had exploited the water treatment devices. The documented history reveal that during the third and the fourth century the Egyptians employed different filtration method to purify their drinking water that techniques which included boiling of to eliminate the microorganisms and removed impurities by filtering it through sand and gravel [5].

### 1.2.3 1627 (A.D.)

Over next several centuries filtration techniques altered very little until 1627, when Sir Robert Bacon made significant innovation in sand filter to distil salt from the sea water. According to him if seawater allowed to drip through the sand, it could be purified. Bacon dug a hole near the shore through which seawater flowed and allowed sand to trap the salts and other particle. His experiment revived the filtration methods and motivated scientists to continue to improve the technology[5]

### 1.2.4 1700 (A.D)

By the 18$^{th}$ century the microscope invention had given scientists new recognition of countless microorganism presence in surroundings and water. In 1854, the British scientist John Snow detected the bacteria which caused the cholera disease and this discovery intensified the research in water treatment .Consequently, many nations began to start utilizing filters made of charcoal sponge and wool to eliminate noxious organisms and particles for health[6].

### 1.2.5 1900 A.D

During 20$^{th}$ century, U.S. realized the utility of drinking water quality and implemented the drinking water standards. Along with US, most developed countries also authorized regulations that set minimum standards of water quality. This persuaded the development of the contemporary filtration techniques we use today [6]

## 1.3 Traditional Tools for Filtration

Filtration is a combination of physical and chemical processes. Mechanical straining expels debris by trapping them between the grains of the filtration medium and some reagents make dissolved particles to suspended form to facilitate the filtration process. Adhesion is an equivalently important process by which suspended particles affixed to the surface of filter grains. Following are the common methods and tools utilized in filtration and water treatment.

*Table 0-1 presents the comparison between common filtration methods*

| | Slow Sand Filtration [7],[8] | Membrane Filtration toxins [9]. [8] | Cartridge Filtration [10], [8] |
|---|---|---|---|
| Method | The filter contains a bed of fine sand generally 3 to 4 feet deep supported by a layer of 1 one-foot layer gravel and drain system. | A membrane is a thin layer of polymer material with pores and capable of straining substances when a driving force is implemented across the membrane. | Cartridge filters are regarded as emerging technology in filtration field. These are classic modular filters that are fitted into a housing. |
| Process | Filters are operated under continuous immersed conditions. Biological processes and chemical/physical processes are executed at the surface of the filter bed- A biological slime referred as Scmutzdecke forms on the exterior of the bed which traps small particles | Membrane filtration is achieved by the passing liquid at high pressure through a thin membrane in the form of hollow fiber. Contaminants are engaged on the high-pressure side and removed frequently by reversing the flow and flushing the remains | Cartridge uses a physical process -to refine water through porous media. it can eliminate particle up to 0.2µm size. |
| Chemicals | This process does not require pre-treatment chemicals like coagulation/flocculation | Frequently backflushing and occasional chemical cleansing is necessary in order to preserve the membrane fibres. | A disinfectant is recommended to avoid surface -fouling and microbial growth on the cartridge surface. Furthermore, anti-corrosive chemicals are required for cleaning and preserving the material. |
| Equipment | Small plants are assembled with cast -in-place concrete structure with wood or concrete slab covers | These are comparatively simple to install and the systems consists of pumps, the membrane modules and storage tanks. | A cartridge is composed of a ceramic or polypropylene filter component inserted into pressurized housings. |
| Advantages | Low cast elementary operation, reliable and accomplish 99% Giardia cyst removal. | Membrane process are effective for removal of bacteria, organic material, microorganisms, and other particulate material. | Cartridge filters are simple to operate and maintain and appropriate for domestic use. . |
| Limitations | Does not convenient for the liquids with high turbidity and requires maintenance regularly | Membrane fouling is the major obstacle in widespread application of this technology. Occasional chemical cleaning is necessary to remove persistent | Cartridges clog relatively quickly therefore must be replaced. They work best for low turbidity water and small volumes. |

## 1.4 Separation based on physical criteria

Separation techniques can also be dependent on the physical nature of the particles such as size, shape, density etc. These techniques are commonly employed for bulk preparation of a heterogeneous sample. In this classification we have membrane filters and centrifugation techniques as fundamental ones.

### 1.4.1 Separation techniques based on size and shape of particles

These techniques are further divided into two sub classes i.e. Direct flow and Cross flow. In Direct flow (sometimes called "dead end") fluid to be filtered is introduced in a direction perpendicular to a filtering surface. Contaminants are trapped or build up on the surface within the filtration media and increase the differential pressure across the filter as it blocks all particles bigger than the pore size of membrane. At a certain limit filtration procedure is stopped and the membrane or filtration media is either discarded or may sometimes be regenerated for re-use by washing it with descaling chemicals [11]. On the other hand, in crossflow filtration configuration the fluid to be filtered is recirculated perpendicularly to membrane surface. The supply pressure drives the decontaminated fluid through the membrane as filtrate (permeate), while bigger suspended particles in the feed fluid are retained in the relatively high concentrated retentate stream Unlike from parallel flow filtration, the solid particles in the retentate do not build up on the membrane surface, but rather they are scrubbed from the membrane surface, which prevents development of dense solid layer on the membrane surface [12].

### 1.4.2 Particle separations based on density

Although density gradient centrifugation can implement for the separation of wide range of particles with diameter less than 20µm, in practice the method has been more confined to the separation of particles from living cells. This technique employs a density gradient medium, a reagent solution that composes of density gradient over the required range. This process involves the centrifugal force for the separation of segments. Relatively denser components of the mixture drift away from the axis of the centrifuge, while less-denser components migrate towards the axis of centrifuge. The effectiveness of the process can be enhanced by increasing the gravitational force on test tube which

will cause more rapid precipitate (pellet) to gather on the bottom of the tube. When the centrifugation is completed, the sample is layered on the media and low-density particles are divided from high-density particles [13].

### 1.4.3 Cell separation techniques based on affinity methods

These methods consists of affinity methods such as capture on affinity solid matrix (beads, plates, fibres) fluorescence-activated cell sorting (FACS) and magnetic cell sorting, which rely on biochemical cell surface characteristics and biophysical criteria (in FACS). The underlying of these methods is given below

#### *1.4.3.1 Chromatography*

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Some of those are: Column Chromatography is a separation technique in which the stationary bed is within a tube. Planar Chromatography is a separation technique in which the stationary phase is present on a substrate. Thin layer chromatography (TLC) is a widely employed laboratory technique and is like paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate [14].

#### *1.4.3.2 Fluorescence-activated cell sorting - FACS*

Fluorescence-activated cell sorting is a particular type of flow cytometry. This technique is applied for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time. Its function is based upon the specific light scattering and fluorescent characteristics of each cell. This instrument is utilized for fast, objective, and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. The acronym FACS is trademarked and owned by Becton Dickinson [15].

#### *1.4.3.3 Magnetic Cell Sorting*

The characterization of specific cell types and the investigation of their functions requires that the cells of interest can be isolated or purified from other contaminating cells. There are several isolation methods available for the specific isolation of cells using nonmagnetic and magnetic separation. Flow-assisted cell sorting (FACS) is a non-magnetic method to obtain highly purified cells. This method, however, is quite time consuming, costly and can be rough on cells. Cell separation techniques based on the use of antibody coated magnetic beads, are now widely used in research and clinical laboratories. There are two types of magnetic cell isolation technologies, column-based and tube-based systems. The column-based technology utilizes smaller, nano-sized particles and therefore requires that the cells be passed through a magnetized iron-mesh column to increase cell-capture capacity. The tube-based system utilizes larger, micron-sized beads. Specific cells can, after binding to the antibody coated magnetic beads, be selected by the use of just a magnet which is held against the sample tube and following brief washing, high cell purity can be achieved [16].

## 1.5 Research Gap

Standard filtration system contains only one system with pores or matrices. The pores or the matrix does not contain specially designed structures or systems. These filters do not do a job either sorting of the particles that are separated or captured. For instance, the microorganisms that transmit disease to humans via water vary significantly in size: protozoa (4-100 micrometre), bacteria (1.0-0.2 micrometre) and viruses (0.1-0.01 micrometre. Secondly , it is possible to physically remove all the microorganisms using different types of membrane filtration but with continuous operation with these membranes choke frequently and their efficiency also reduces if the operation fluid is relatively more impure .Similarly, in a differentiated sand filter, just about the particles captured without being able to be transported on as finished purified particles. Moreover, most filter systems used in seawater or on difficult complex liquids will quickly become clogged especially if they have to treat large volume of liquid. Moreover in many filtration system the treatment with the chemicals are mandatory to avoid scaling in system or for the coagulation purposes .This step may change the nature and behaviour of the biological fluid and make difficult to perform the analysis on particles.

## 1.6 Emergence of microfilters

While filters have existed for a long time, but the concept of micro technology, micro fluidics and micro-filters is still in a developing phase. The first transistor was patented in 1925 by Physicist Julius Edgar Lilienfeld in Canada, but it was not until 1950 that the research sped up and development of transistor began for real and started the era of microtechnology [17]. This has evolved from one transistor to today´s oodles of transistors on a single chip. Along with micro technology there has been a progress within combined field of micro technology and fluid mechanics called microfluidics. In microfluidics there has been developments. From the 1950s attempts were made to dispense small amount of liquid which led to today's ink printer which can dispense pico litre of liquid. The development has emerged enormously from the 50s and up till now. In 1990s the concept of µTAS (micro Total Analysis System) that latter became LOC (Lab on a Chip) and brings up the developments within the area of biomedical research.[18] The development was accelerating together with the field of transistors, biology, surface chemistry etc there was a drive to make things smaller that led the way to a new segment, nanotechnology.

## 1.7 Problem statement

Most microfluidic devices use separation or isolation methods function based on different mechanisms, including filtration, hydrodynamic filtration, deterministic later displacement (DLD), inertial forces, deterministic later flow, pinched flow fractionation, acoustic separation, dielectrophoretic (DEP), magnetic, electrokinetic and optical forces. Each microfluidic strategy possesses certain advantages and disadvantages. The following main issues were highlighted in traditional microfiltration methods and main goals were selected for the subsequent project.

- Microfilters cannot accommodate the large volume and prone to clogging. They operate usually in ml/min range
- Microfiltration is often carried out in several manual steps and not automatic
- Microfilters are often not suited for up-scaling

## 1.8 Research Objectives

Various complex and heterogeneous liquids, such as bio-liquid, oil, sludge, and seawater, industrial- and wastewater, contain valuable resources and information for industry and society. Our world contains interesting unexplored or unused minerals, macro-polymers, macromolecules, proteins, microorganisms. Sustainable use of these resources, requires particles that must be separated, concentrated, and refined into a manageable volume of high purity and concentration. The main challenge is how to handle the size, shape, content, surface and weight of elements or particles found in complex or heterogeneous solutions, gases, or solids, using sustainable production or technology. The PASECOR centre will solve the handling and sorting of both large and very small particles, refining the largest particles first to be able to further refine the small particles. This will generate new knowledge in the understanding within physics, microfluidics, analytics, chemistry, and biology. Through PASECOR it will be make possible continuous separation, sorting and concentrations of the particles based on nano technology fabrication methods which will do the operation regardless the size of the particles to be separated and volume of the fluid to be handled. To achieve these goals the objectives were short listed for the following project

- Design a microfluidic chip to handle and sort the large as well as very small particles
- Fabricate the chips by employing micro/ nano fabrication techniques
- Assemble the chips to evaluate the performance

## 1.9 Limitation & Scope

Although these microfluidic chips utilize the micro/nano fabrication methods and techniques but in this novel project we had not touch the limits to separate the smallest possible particles in nano size range. Instead it would focus on the particle which are in range of 5 micron initially. Secondly, as this PASECOR technology is in a developing phase and all the research is still carried on a laboratory scale therefore the practicality of the project yet to be determined on the large commercial scale.

## 1.10 Thesis Outline

- Scientific literature and introductory theory of the PASECOR technology is discussed in chapter two.
- Design and layout of PASECOR chips are portrayed in chapter three.
- Methodology of fabrication process is explained in chapter four.
- Results of the fabrication process are displayed in chapter five.
- Issues related to the fabrication process are discussed in chapter six.
- Future work and conclusion are illustrated in chapter seven.

# 2 Review & Theory

## 2.1 Literature Review

The idea and concept of microfluidic based filtration devices has already been presented in many of the scientific articles and research papers particularly since last two decades. Researchers has presented their own approach and design to prove it feasibility. They have made drawings lay outs, build geometries, performed simulations, and even they have been using injection moulding or 3D printing method to build a prototype and tested its performance. Some of the research work, findings and limitations are highlighted in following sections [19] .

### 2.1.1 Integrated micromachined separator

A smart fully integrated micromachined separator with soft magnetic micro-pillar arrays was investigated. The study was performed to examine design , simulation and experiments to verify the capacity of the micro separator by isolating the lymphocytes, in which the human whole blood mixed with DynabeadsR Flow Comp Flexi and monoclonal antibody MHCD2704 was used as the sample. The simulation results demonstrated that micro-pillar arrays escalated the electromagnetic field generated by the micro-coils. The experimental results discovered that the micro separator trapped the target particle lymphocytes with a high efficiency. The study focused mainly on the simulation and modelling and need to prove the results in laboratory [20].

### 2.1.2 Turbine micropillar microfilter

A study was conducted to reduce the clogging in microfilter for blood cell (erythrocyte, leukocytes, and epithelia) and presented the turbine blade-like micropillar were introduced in microfilter design. The research was based on simulation in two different designs which employed, first one with varied cross-section channels, and the other with Archimedes' spiral channels. The simulations-based data verified that both microfilters designs had enhanced separation efficiency and incredible anti-clogging characteristics. it also determined the optimum angle between the penetrate flow and main flow which must be greater than 0° and found that larger angles are more effective. The study also found that counter flow is better than cross flow [21].

### 2.1.3 Non-clogging micro concentrator

Another study was conducted to demonstrate the integratable non-clogging micro concentrator based on counter-flow principle for continuous enrichment of CaSki cells. In this study, a counter-flow unit was introduced to elaborate a micro concentrator/microfilter for the CaSki cells, by arranging the streamlined turbine blade-like micropillars into the ellipse-like structure. The framework and the parameters of the counter-flow unit were optimized with CFD (computational fluid dynamics) simulation methods. The study revealed that the concentration characteristic of particles depends on the volumetric flow rate which established that a lower volumetric flow rate assist to a higher concentration ratio for the separation. The visualized experiments validated that no cell-clogging phenomena occurred during the test and that no cells were found in the final filtrate. The study was conducted on the small volume and more work need to do to accommodate the large volume of fluid [22].

### 2.1.4 Non-clogging counter-flow micro concentrator

In another research an analysis was performed to investigate the design and optimization of non-clogging counter-flow micro concentrator for enriching epidermoid cervical carcinoma cells with turbine blade-like micropillar which were introduced in micro concentrator design. Typical microfilters were also examined that provide good efficiency on cell concentration. The design of the device was optimized by coupling Computational Fluid Dynamics (CFD) and Artificial Neuron Network (ANN). The experimental result proved that it did not clog even at permeate entrances. The flow characteristics of the filter units were examined which authenticated both simulation and experiment results. Study also endorsed that the turbine like pillars provide a unique geometrical-profiles and fantastic microfluidic efficiency. The focus of the study was on design optimization by simulation methods. The concept has still been tested on more complex biological fluidics to improve this design and fabricate the device [23].

### 2.1.5 Continuous separation of microparticles

In this article the authors conducted a research to study the clogging-free microfluidics for continuous size-based separation of microparticles and introduced a lateral flow microfluidic sieving (μ-sieving) structure to lower the clogging and to allow continuous separation of micro particles based on microfluidic isolation. A low frequency

mechanical oscillation was included to the fluid flow by a piezoelectric actuator which promoted the release of undesired polystyrene (PS) particles captured between the bigger target PS particles. The experimental results found that PS particles of 20µm were successfully isolated from 5 MPs particles and exceptional recovery rate were also observed as more than 98% of the separated particles were recovered. The same technique was applied to separate the cancer cells (MDA-MB-231) from whole blood and observed that the fluidic oscillations intercepted the filters from being clogged and allowed continuous microfluidic operation with high performance. The device has the processing layer and there is no information regarding the collection of the particles. It was fabricated with micro-pillars had intervals of 12 µ m for the polymer microsphere sieving which limits the applications [24].

### 2.1.6 Micro particle separation through membrane

In this report the author presented a different approach to elaborate a clogging-free and bubble-free micro particle separation mechanism with high efficiency and throughput. The mechanism consists of an integrated bidirectional micropump, a hydrophilic microporous filtration membrane and a hydrophobic porous degassing membrane. The study was conducted on microbead mixture and undiluted whole blood. The results accomplished the 90 % recovery rate of 10 µm microbeads and 70.6% efficiency for white blood cell. The membranes clog in between and must refresh which increase the operation time and the further study need to be done to separate micro particle under 10 µm range.

## 2.2 Vision of PASECOR

The University of South-Eastern Norway has the plan to integrate a Centre for autonomous Particle Separation, Concentration and Refining (PASECOR) inside the new USN Innovation centre. The intent is to resolve the challenges for purify or decontaminate the complex fluids from impurities and pollutants through research and innovation. The ultimate objective is to separate, concentrate and characterize with high accuracy regardless the complexity of the mixture and size of the particle to be separated ranging from macro to molecular. Through this technology we can combine devices constructed to perform physical and continuous separation into a system for refining of all particles

inside the solution. The complex fluid addressed within PASECOR are oil/ water , water with high number of different particles , waste water , cloak water , drinking water , liquid biopsy fluids, fluids with fatty acids ( triglycerides or lipids ) fluids with polymer additives , whole blood , serum , water/soil mixture , water with hard particles etc. The micro particles also vary in sizes for examples the microorganisms like algae (4-100 µm), bacteria (1-0.2 µm) and viruses (0.1 -0.01µm) and all other toxins present in fluid (air/liquid) , lies in scope of PASECOR .

### 2.2.1 Applications of PASECOR

The knowledge and innovation generated by PASECOR can contribute to solve challenges and address important issues related to one or more of the following UN's sustainability goals promoting more sustainable value creation and sustainable restructuring of the Norwegian industrial sector. Some of the areas addressed within PASECOR are highlighted below.

The possibility to separate, concentrate and refine particles from oceans, rivers, lakes, and fish farming slaughterhouse can be used to generate refined biomasses and very nutrient rich purified foods, micro animals, and small fish. Most of these resources are not in use by any society in the world. Therefore, it may be used to fulfil food needs by developing very sustainable industries, aquaculture, and fisheries.

1. The possibility to separate, concentrate and refine particles and micro animals in organic or blood related fluidics may dramatically increase the accuracy of diagnostics. The significant increase in clinical accuracy make it also possible to perform preventive health diagnostics or treatment everywhere. This may in turn reduce the overall cost for health services for society.
2. PASECOR technology provides us opportunity to obtain particles or elements in organic fluidics and will facilitate higher number of high-quality drugs. Many particles, macro proteins, biopolymers or polysaccharides may at the first time being able to undergo research and production. This will open the door to many new personal medicine-based technologies.
3. By making it possible to perform direct physical and continuous separation, concentration and refining of all kinds of water make it possible to produce high quality and very pure drinking water in a very sustainable way.

4. The possibility dramatically removes and refine dust and particles in large volumes of water may be used to reduce the destruction of turbine blades and at the same time concentrate particles that is important for the biological and agricultural industry.
5. Large ocean industrial infrastructures are producing polluted industrial waste and therefore reduce the overall sustainability of the production plant. The possibility to remove pollutants from sludge will produce useful commercial products.
6. PASECOR technology can be applied to reuse wastewater and runoff produced by a city.
7. Large agriculture fields are not able to be used in a sustainable way due to lack of control of bacteria and microorganisms in biological and surface waters or liquids. The possibility to separate, concentrate and refine these kinds of liquids may dramatically increase the control and growth of all kinds of agricultural crops, fruits, and drug production. The digital and molecular biological control of large areas of natural and artificial forests using PASECOR technology inside agriculture robots or following pooling of surface waters or waters from saps may improve the management and increase the possibility for more sustainable growth and fruit production.
8. PASECOR technology will enable monitoring of known and unknown life below sea, by the direct and specific concentration and refining of particles, elements or micro animals or marine species of interests.

## 2.3 Scope of Work

The main goal of the proposed project was separation of bacteria (1.0 -0.2 µm) from the raw oil solutions. By handling or treatment of large volumes of liquids could be optimized and develop cost efficient assembly methods of high volume of micro and nano fabricated devices. It will help to reduce the cost of environmental control, make the environmental control more accurate and find the area with possible high-quality oil. However, the first target of the project was to separate and concentrate microalgae (2.0-10.0 micrometer) from ocean water, lake water and raw oil solutions then it would be tested to separate and concentrate all other microorganisms present in crude oil.

### 2.3.1 Oil eating bacteria

The ocean is home to many groups of bacteria that can break down the chemicals found in crude oil. Some, like Alcanivorax, are oil-eating specialists that are usually found in low numbers, which bloom only when oil spills provide them with a sudden banquet. Scientists have found that these oil-eaters are further divided in sixteen groups and these swelled in number in the contaminated waters. All sixteen groups have members that can digest the hydrocarbons in oil and can do so in very cold environments where most bacteria would grind to a halt. These plume bacteria were genetically distinct from those outside and their growth is independent of temperature, salinity, and pressure [25].

### 2.3.2 Framework and Execution

The project allowed the university to produce PASECOR chips ideal for the treatment of oil-eating bacteria to be added to the pilot test rig of PASECOR. The PASECOR Centre has already completed the TRL(technology readiness level) level 1 to 3 in which the studies and research has been conducted on different transport mechanism, liquid behaviour, gas-liquid technology, surfaces, polymer materials, particle behaviour, bonding and packaging processes and also some prototypes have been made .The purpose was to go from TRL 3 to TRL 5 to prove the concept, where bacteria were separated from a complex fluid (Oil). In order to prepare for TRL level 5 the university combined photolithography with reactive Ion Etching (RIE) method to make the structure on both side of the PASECOR chip.

Photolithography is the process by which a light source (Ultraviolet) is typically used to transfer an image from a patterned mask to a photosensitive layer (photoresist) on a substrate. Photolithography performance is determined by minimum feature size that can be transferred with high fidelity, its resolution, the throughput and how precisely patterns on successive masks are aligned.

In Dry etching process, the protective layer (photoresist) which is deposited during photolithography identify the material to be removed and protect the material that is not to be etched. Dry etching offers excellent process control for cleanliness, homogeneity, etch-rate, etch-profile, selectivity, and run-to-run consistency, which is critical for high-fidelity pattern-transfer in micro- and nano-system technologies. Reactive ion etching

utilizes moderate level of RF power and pressure to combine both physical and chemical etching in one process .RIE has further subclass named as DRIE (Deep reactive ion etching ) is used to etch trenches or deep cavities in substrate with high aspect ratio ( the ratio of cavity depth to its width . The RIE also ensures isotropic and anisotropic material removal which means we have more and better directional control [26].

In addition, the USN has ideal bonding equipment in order to cover both sides of the structure of the PASECOR chips with glass plates which will be carried out by Anode Bonding. Anodic bonding is a solid state, irreversible bonding technique by electrostatically bonding two dissimilar materials together (glass, metals, alloys, semiconductors) having a coefficient of thermal expansion (CTE) close to each other .It will secure connection with microfluid channels in and out of the included chips. Finally, in order to verify quantity and type bacteria in an oil solution, 3th generation of sequencing is necessary of the characterization of bacteria.

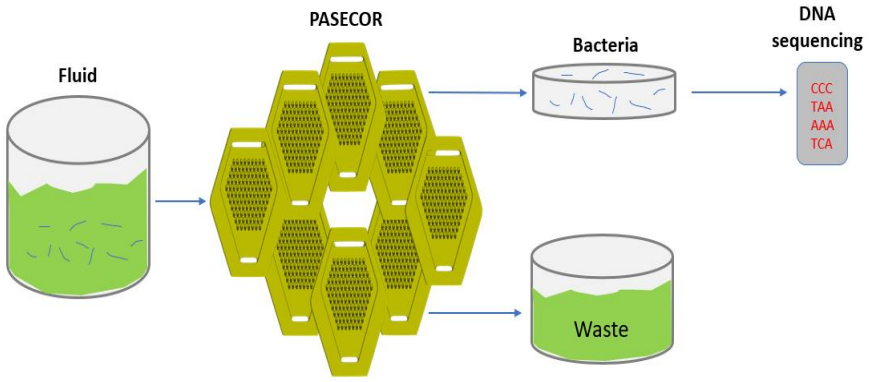


*Figure 2-1 highlight the working of the PASECOR chip from raw fluid to sequenced data.*

# 3 Design and layout

## 3.1 Designing Task

For particle separation , one of the most important research tasks to secure that particles defined as cells, bacteria, minerals, virus, nanoparticles, microalgae, macro proteins, elements or macropolymers may not be modified, destroyed or removed in manual or automatic operations. One of the most complicated and challenging tasks, during development of complex liquid refining systems, is the construction of arrays of micro- and nano- pillar structures using suitable materials and having desired surface chemistry which can perform physical separation of particles smaller than 25 micrometres .

At present, PASECOR technology has no obstruction regarding type of particles that can be evaluated. Elementary size limits for particles that can be separated with refining technology stretch between 40 nanometres and 1 000 000 manometer/1 mm. The purity confides in size, weight, composition, density, surface and shape of the particle to be isolated and concentrated. If the mixture does not contain other particles with the same characteristics as the target particle, we achieve almost 100% separation efficiency.

## 3.2 Design description

PASECOR chip incorporate multitude of refining unit and micropillars systematized on elliptical profile. The optimized elliptical outline ensures a paramount velocity zone along the two sides of the ellipse. This high velocity region directs the prevention of particle clogging at the penetrated channels. The continuous separation units (CSU) are arranged relative to each other in a continuous separation as shown in figure 3-1 and concentration unit (CSC) in a chamber that has either one or two inputs and 2 outputs, in order to execute optimal separation and concentration of cells or particles in a non-condensing (non-waving) manner (CSCIF). The CS units can be designed in such a way that it is possible to perform first the continuous isolation of the largest particles in this way all particles in heterogeneous mixture can be concentrated , purified and isolated in different sections according to size, surface, shape, content and weight furthermore they can absolutely identified or analysed. The design of the chip assures that suspension does not bubbles, or gas / liquid phases appear which reduce effectiveness of the whole separating operation.


*Figure 3-1 shows the structure of the main middle layer and elaborates the design of chip*

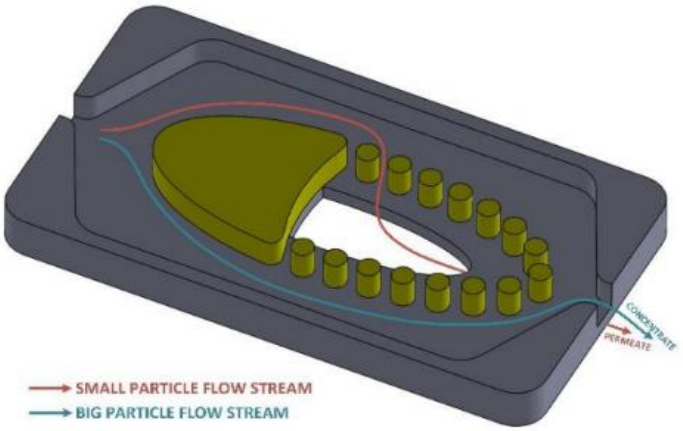


*Figure 3-2 describes the flow of fluid and particles through microfluidic continuous separation units*

The actual device consists of three layer as shown in figure 3-3 , the top and bottom layer contains connection for lure plugs so that we could make a network of flow channels through tubing. The liquid will enter from the center of the top layer toward down toward the middle layer and pass through CSP and CSO units as shown in 3-1. The liquid with bigger particles than the gap between the pillars (concentrate regime) will not pass through the pillars and eventually will come out from the outlet aligned in top layer as shown in figure 3-4 via tubing connection and on the other hand the liquid with smaller particle than the gap of the pillars will pass through to the bottom layer ( permeate) and eventually collected from the bottom via concentration units as shown in figure 3-5.

There are certain challenges like outlet flow channels must be designed such that there would not any accumulation of liquid or particles inside the device and the operation must be carried out in a continuous manner without drop in pressure. These challenges will define the flow rate, inlet/outlet area for the fluid flow as well as the number of CSU/CSC, the distance between the pillars and the number of pillars. In order to address these challenges, there were certain mathematical work based on fluid mechanics which is presented in the following section.

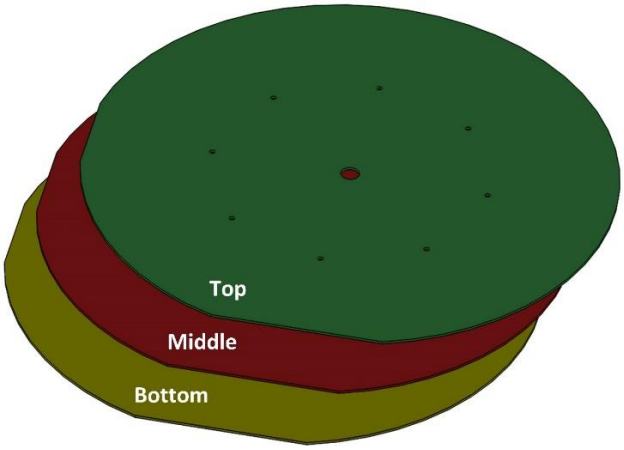


*Figure 3-3 describe the layout for assembling of the device*

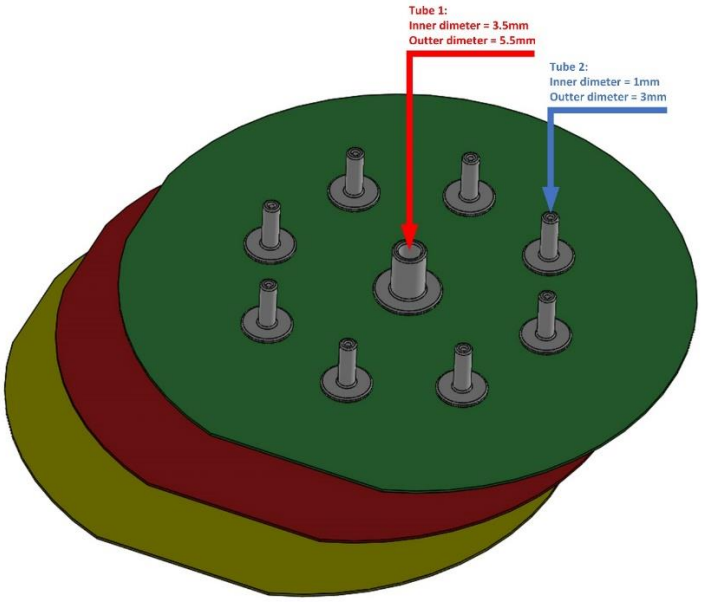


*Figure 3-4 shows the integrating of Luer plugs on TOP layer*

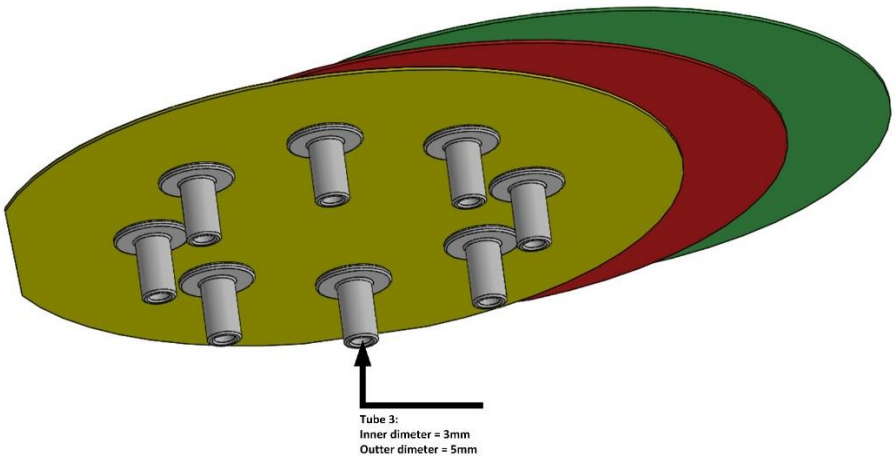


*Figure 3-5 shows the integrating of Luer plugs on Bottom layer*

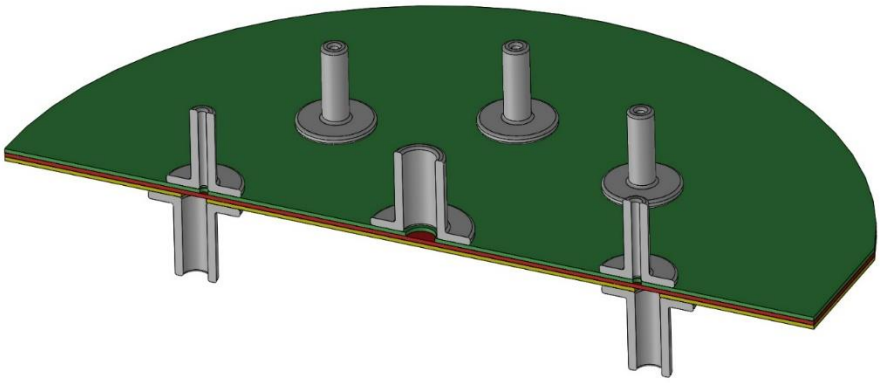

*Figure 3-6 Cross section of assembly with three layers and luer plugs*

### 3.2.1 Continuity principle

When we design a new microfluidic chip , it is important to take into account the continuity principle which states that the mass flow rate into the system must equal the mass flow rate out from the system .The mathematical expression and simple layout of based on continuity principle is shown in the figure below

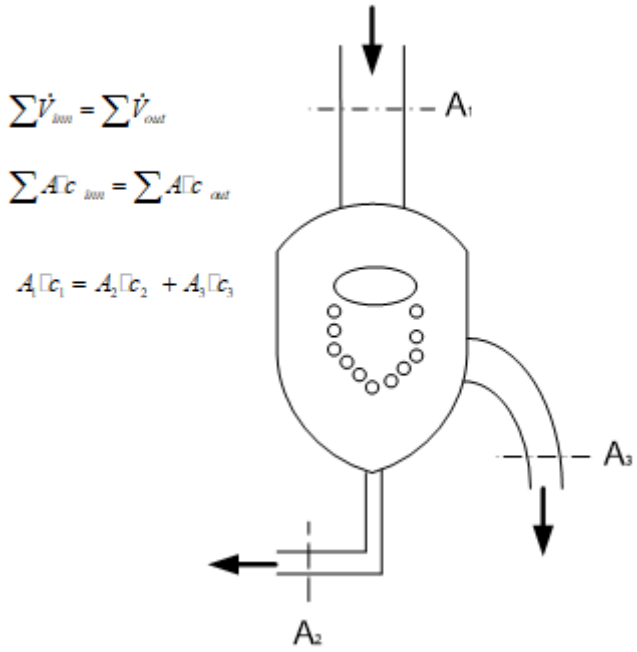


*Figure 3-7describes the general continuity principle*

*Some useful expressions regarding chip designing are*

- $A_{CSU}$= (Height of pillar) (Distance between pillars) (Number of gaps)
- $A_{CSC}=A_{CSU}$. (number of CSU)
- Acsc should be equal $A_3$ in our chip

Important to take into account that 80% of the volume flow should follow a specific outlet channel, and 20% of the volume flow in a second outlet channel.

### 3.2.2 Calculations

In this novel project, the target liquid volume is 80% mixed with the smallest particles(concentrate) then cross-sectional area through all channels inside the chip must take this into account. The flow velocity (c) in all channels should be the same, the cross-sectional areas through the two outlets must account for this.

The important tasks for design are enlisted as

- The inlet: What should be the optimal cross section area and the optimal flow velocity?
- The two outlets: What should be the optimal cross section areas and the optimal flow velocities?

So which flow velocities do we need both in the inlet channel and in the two outlet channels.

In order to design the chip, we assumed the total inlet flow rate of the liquid was (10 ml/min = 0,1667 ml/s). then for

Inlet

- volume flow rate = 0,1667 cm3/s
- area of chip = 0,2 cm2 (100%)
- velocity of chip= 0,833 cm/s.

Outlet

- volume flow rate = 0,1667 cm3/s

since there are two outlet channels (permeate and concentrate) and velocity was considered constant, then

- area of permeate outlet = 0,2 cm2 * 0,8 = 0,16 cm2 (80%)
- outlet velocity of permeate = 0,833 cm/s.

and

- area of concentrate outlet = 0,2 cm2 * 0,2 = 0,04 cm2 (20%)
- velocity of concentrate = 0,833 cm/s.

The speed in the inlet is 2 x the speed in the two outlets:so

- The outlet area of the permeate= 0.32 cm2 (80%)
- Speed of the permeate= 0.4165 cm / s.
- The outlet area of concentrate= 0.08 cm2 (20%)
- Speed of consent rate= 0.4165 cm / s.
- If volume flow into the chip is 0.1667 cm3 / s.
- The inlet area of the chip is Ain = 0.2 cm2.
- Speed into the chip is Cin = 0.833 cm / s.

Of the volume stream sent in, 20% of the liquid should follow the concentrate, while 80% should follow the permeate. The volume flow will then be distributed as follows:

- Ain * Cin = Aconc * Cconc + Aperm * Cpermt
- 0.1667 cm3 / s = 0.03334 cm3 / s + 0.13336 cm3 / s

If suppose number of CSC units / chip is set to be 23 pcs and each CSC unit consists of 15 pillars, with a distance of 25 µm between each pillar (ie 16 gaps / CSC unit, each gap with a width of 25 µm and a height of 100 µm).

- The area the permeate will pass through per CSC unit will here be (16 * 25 µm * 100 µm) = 0.04 cm2.
- $A_{in} * C_{in} = A_{conc} * C_{conc} + A_{perm} * C_{permt}$

  0.2 cm2 * 2.5 cm / s = 0.1 cm2 * 1 cm / s + 0.04 cm2 * 1 cm / s

### Case 1

When there are 23 CSC units / chip,

- the total area of the permeate will be 23 * 0.04 cm3 = 0.92 cm3
- The outlet area connected to the permeate must here be 0.92 cm2. With a defined height of 0.1 cm, an outlet width of 9.2 cm must be designed.
- The outlet area for the concentrate must be 0.1 cm2
- The flow rate in both the two outlet channels will be 1 cm / s

If the outlet area of the permeate is to be reduced, then the number of CSC units per chip should be reduced. Let if the height is increased to 0.2 cm, then the width of the outlet channel will be 4.6 cm.

### Case 2

When there are 16 CSC units / chip,

- the total area of the permeate will be 16 * 0.04 cm3 = 0.64 cm3.
- The outlet area connected to the permeate must here be 0.64 cm2. With a defined height of 0.1 cm, an outlet width of 6.4 cm must then be designed.
- The outlet area for the concentrate must be 0.1 cm2
- The flow rate in both the two outlet channels will be 1 cm / s

If the outlet area of the permeate is to be reduced, then the number of CSC units per chip should be reduced. Let if the height is increased to 0.2 cm, then the width of the outlet channel will be 3.2 cm.

## 3.3 Available Fabrication Techniques and limitations

The fabrication of the units relies on the already established refining chip technology protocols and is accomplished using microfabrication techniques. Currently Injection moulding and hot embossing are the preferred methods for fabricating microstructures with high intensity of precision, accuracy, rigidity, and shape uniformity. These means are employed to produce hard enough pillars, straight enough surfaces and structures that can withstand high pressure, does not produce pressure loss or create air bubbles.

Through injection moulding the CSC units are built on all types of plastics materials to treat all particles fluctuating in size between 1-10 micrometre/1000-10 000 nanometre. The refining chips can be fashioned on all types of materials that can tolerate chemical treatments so that we could engage micro or nano fabrication strategies like photolithography and etching. Consequently, this will secure the continuous separation of particles less than one micrometre. Typically, for the particles which lie between 1-50 microns size it is possible to consume plastic materials and utilize injection moulding techniques. These units can be produced in bulk and reduce the financial cost significantly. For the particle under a micrometre, the Continuous separation units emerged so small that silicon or other similar substrate are adopted for direct micro or nano fabrication.

## 3.4 Design Modification

in the novel project the same concept of the micro particle's separation is addressed with some improvements in design and the structure. Instead of introducing the turbine like micro pillar, cylinder like verticals pillars are incorporated. The concept of turbine like blades have been trial and tested with injection moulding and it was found that it had some shortcomings. Despite that design posing excellent flow characteristics due to their aero dynamic principle but they offer significant drop in pressure when the flow rate was increased.

## 3.5 Features of chip

The PASECOR chip structure generally compose of a processing layer (middle layer) and a collection layer (bottom layer). The processing layer (middle layer) comprises of the

inlet, the plurality of continuous separation units and the outlet. The collection layer (bottom layer) is constituted of a profusion of collection channels for incoming fluid, from channels of the separation units and the outlet. The mechanism may further involve a cover layer (top layer) above the processing layer to ensure fluid-tight sealing. The transport characteristics of the processing layer rely on the amount and the dimensions of the separation units. The Optimal surfaces are created by using monolayers, to ensure superior surface hydrophilicity. The pre-treated fluid will be supplied from one end of the wafer stack driving all the liquid pass through the whole microfluidic system. The specification of each layer is given below.

### 3.5.1 Top layer

The top layer will be made of glass which has low expansion ratio and contain through holes. The flat polished sheets are used as sight glasses, windows. This layer must be transparent because we want to observe the flow of the fluid inside further it will facilitate tight sealing. The top glass is imported from Germany and the specifications of the glass are tabulated Table 3.1 as,

Table 2: *Table 3.3: presents the particulars of the top layer fabricated on glass*

| | |
|---|---|
| Material | Borofloat33 |
| Diameter | 100.0 ±0.3 mm |
| thickness | 900 ±10 µm |
| Polish | double side with two flats (32.5 and 18.0 mm) |
| Temperature withstand | Up to 450 °C |

### 3.5.2 Bottom layer

The bottom layer is realized on the glass and has holes and pattern which form a microchannel network that permits to maintain a certain pressure and to avoid the inversion of the flow through the downstream hole. 752 pillars are to form the microchannel network in the bottom layer. The bottom wafer is also fabricated in Germany and the specifications of the bottom layer wafer are tabulated in Table 3.3.

*Table 3.3: presents the particulars of the bottom layer fabricated on glass*

| | |
|---|---|
| Material | Borofloat33 |
| Diameter | 100.0±0.3mm |
| thickness | 900 ±10 µm |
| Polish | Single side |

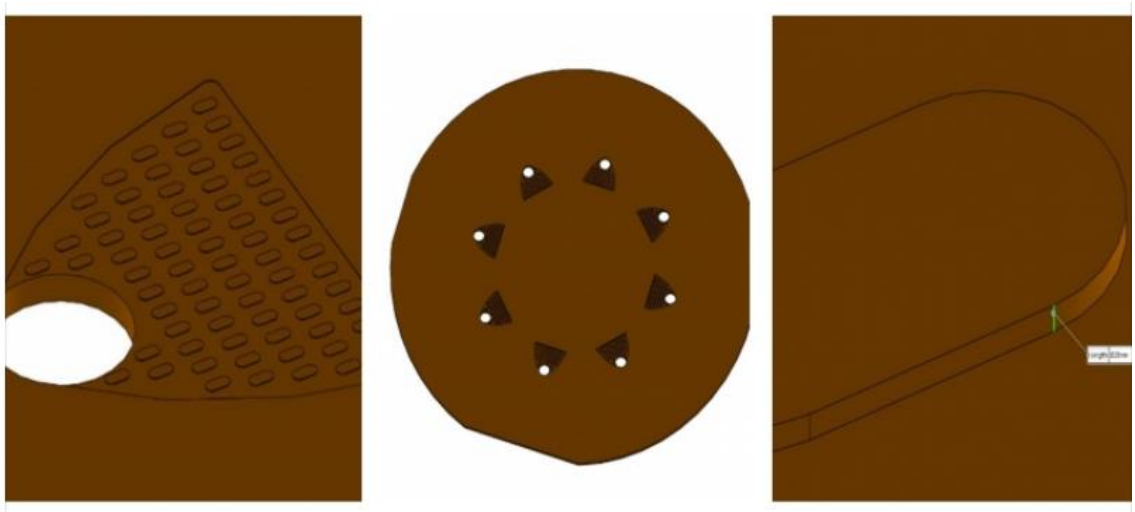

*Figure 3-8 shows the structure of bottom glass layer and integrated holes*

### 3.5.3 Middle layer

The middle layer is the processing layer and is vital part of PASECOR chip and is realized on silicon substrate in IMS clean room .It constitutes the filter units as described in section 3.2. The middle layer will be fabricated by using microfabrication techniques, lithography, and deep reactive ion etching (DRIE).

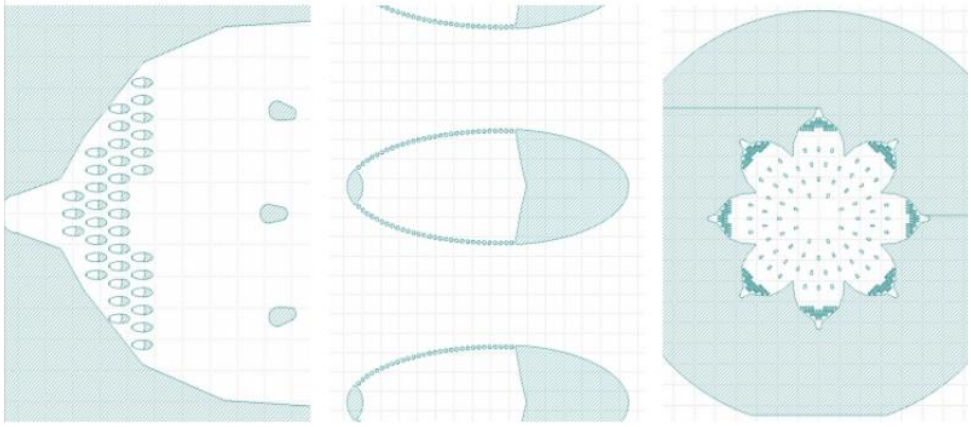

*Figure 3-9 shows the layout of the middle layer*

## 3.6 Design of the Masks

The middle layer is realized on silicon wafer here at IMS clean room and for that purpose two masks were designed. The middle layer is fabricated mainly in two steps and therefore mask for each layer is defined separately one has 10µm gaps and the other has 12µm gaps. The masks of each layer are designed in L-edit and the images of the masks are shown in figures

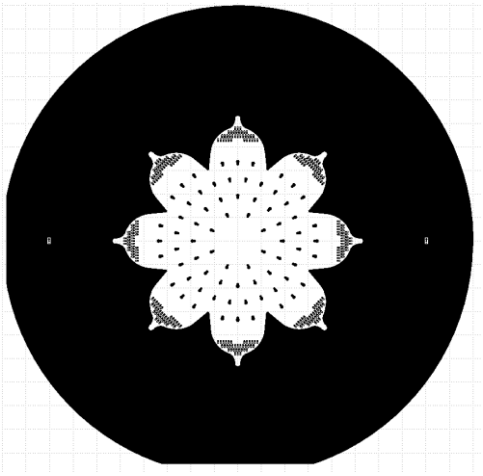

*Figure 3-10 shows the design of the first mask which contains micro pillars and flow channels*

In the figure the first mask is displayed which will define the whole geometry of the structure. it shows the 8 sections of the structure which contains the various separation units. These units are not very much visible in the figure. These units are shaped in an elliptical contour and consists of micropillars

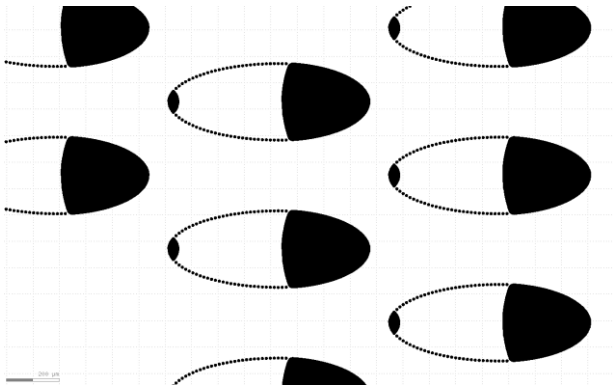

*Figure 3-11 shows the design of the mask in which the micropillar are arranged in the elliptical profile*

This figure 2 Clearly shows the elliptical profile with micropillars. the distance between these micropillars are 5micron. the height of the pillars will be patterned as 10micron by etching the silicon substrate. The inside region of the pillars will be etched through by employing 2nd mask.

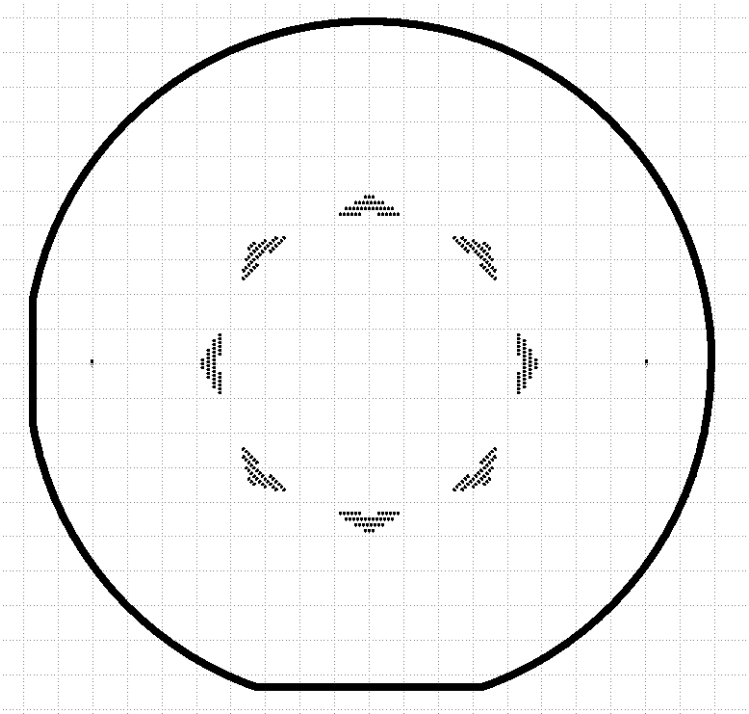

*Figure 3-12 shows the design for the 2nd mask used in photolithography to create holes*

Figure 3 shows the second mask which will used in 2nd lithography to create the holes on the empty region of the ellipse.

*Figure 3-13 shows the alignment marks on the mask 1 used in first photolithography*

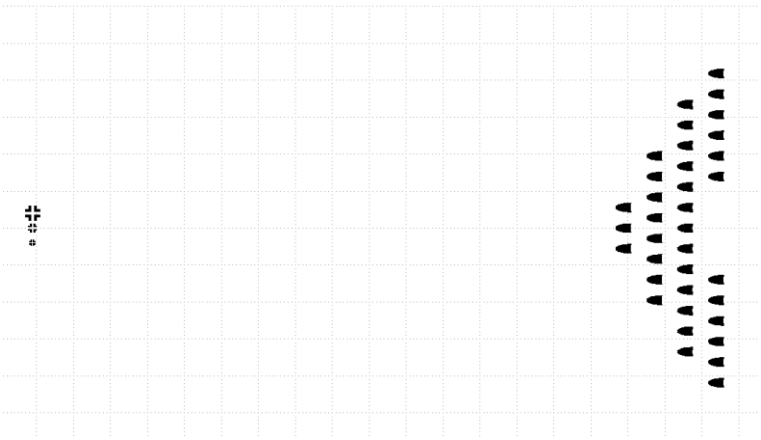

*Figure 3-14 shows the alignment marks on the 2nd mask used in second photolithography*

Figure 3-13 and 3-14 shows the typical alignment marks on the masks. In order to do perfect alignment, the plus sign symbol in the first mask must be fit inside marks patterned on the second mask.

# 4 Methods

## 4.1 Process outline

The middle layer of the microfluidic device was realized on silicon substrate in IMS clean room of class 1 and the glass wafers were fabricated in Germany.

The silicon wafers which were used during fabrication have the following specifications

*Table 4-1 describe the features of silicon wafer used in the fabrication process*

| | |
|---|---|
| Orientation | <100> |
| Polished | single sided |
| Diameter | 100mm |
| Thickness | 525 ±25µm |
| Resistivity | 1-30 ohm.cm |

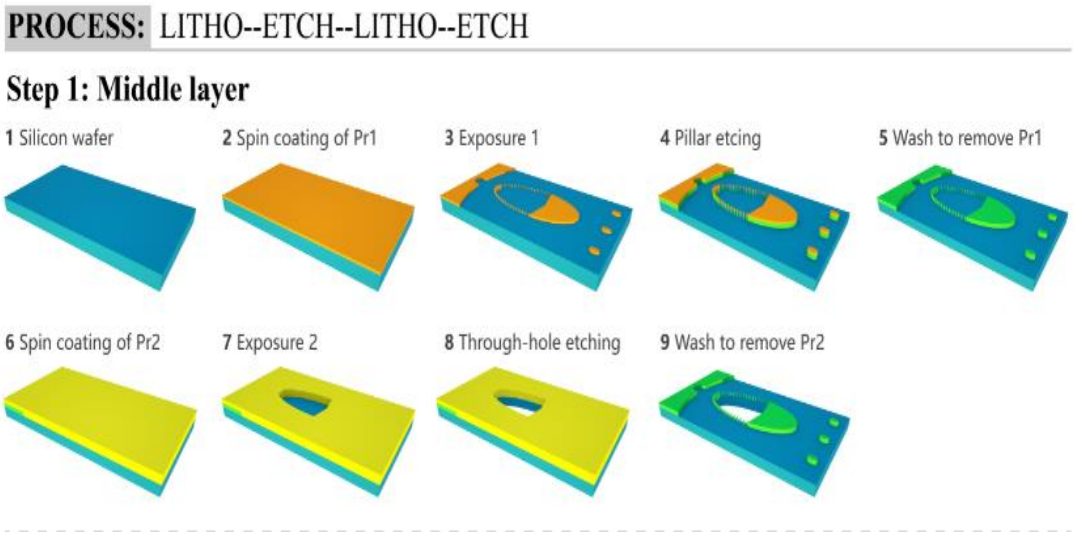


*Figure 4-1describe the outline of process step which were followed during fabrication on silicon wafer*

## 4.2 First photolithography with positive resist

The initial process was to do photolithography with positive resist (PR) S-1813.

Photolithography was being performed in three main steps

- Surface conditioning and Coating
- Exposing
- Developing

The details of every step are described as below

### 4.2.1 Surface Conditioning and coating

#### *4.2.1.1 Cleaning*

It is generally the very first step in lithography process and surface conditioning. For the initial trials the wafer were cleaned manually by putting the wafer in wafer holder and washed it with solvents like Acetone and Isopropanol. Then wafers were further washed and cleaned with demineralized water to ensure detachment of any fragment on the wafer surface then it was dried by high pressure dry nitrogen gas.

Later, to enhance the cleaning process an ultrasonic bath cleaning method was incorporated which is a more appropriate wet method to promote the cleaning process. In an ultrasonic scrubbing wafer was soaked in a beaker for 10 minutes with each solvent (acetone and isopropanol). This agitation mitigates the residues from the wafer which was later scrubbed completely by demineralized water and finally it was blow dried with nitrogen gas.

During the photolithography process it was observed that some wafer did not yield the desired results so the cleaning process was more emphasized and after washing with chemical reagents a dry cleaning method also employed where wafers were placed inside the Oxygen Plasma cleaner( Alpha Plasma AL 18) for extra cleaning. A generator of 200 W and oxygen pressure of 200sccm was used for 5-10 minutes to secure that substrate did not contain any kind of pollutants, unpleasant substances, and stains.

#### *4.2.1.2 Dehydration*

After the wafer was washed it was baked on hot plate for 30 minutes at a temperature 130 °C so that any water or liquid molecules present could be evaporated, and we got an exterior without any moisture contents. Then wafer was let to be cool down at room temperature for 5-10 minutes.

#### *4.2.1.3 Coating*

After dehydration and cooling down at room temperature , a thin layer of positive photoresist S-1813 was deposited on the wafer surface .To achieve this a resist spinner (Photoresist spinner AB plast spin 150 ) was used and its major purpose was to distribute

the photoresist on the surface of the wafer uniformly such that we got a specific and required thick layer(10-25µm) of photoresist.

*Table 4-2 shows the spin program which was operated to get the required film thickness*

| | Speed | Time | AC |
|---|---|---|---|
| Step 1 | 1500rpm | 60s | 1000 |
| Step 2 | 1800rpm | 20s | 1000 |

*Procedure*

Spin coating is the most used method to coat the wafer with photoresist. The following. steps were performed during the spin coating

- The wafer was placed on the vacuum chuck such that the wafer center is on the center of chuck. Vacuum chuck holds the wafer during spinning and avoid it to fell.
- The photoresist was dispensed by using a dropper or small glass bottle such that it could spread about half of the wafer surface as the too much resist causes hillocks , ridges and too little resist may not spread fully and cover the whole surface .
- The spinner lid was closed then we started the selected program for spinning. The chuck accelerates and spread the photoresist across the entire surface of the wafer.
- The excess resist was thrown off on the walls of spinner and we got a uniform layer of the resist
- The photoresist was let to stay on the surface of the wafer so that solvent present in the mixture could evaporate from the surface.

#### *4.2.1.4 Soft baking*

After the application of resist wafer was soft baked for 2 minutes at a temperature of 115C$^{o}$ to evaporate the residual solvents. After soft bake, the wafer was cooled to room temperature.

### 4.2.2 Alignment and Exposure

This step was done in wafer aligner EVG620. After the soft baking, the resist coted wafer was set into an exposure system where they were aligned with glass mask which was inserted first in the aligner. A typical wafer aligner composes of an ultraviolet lamp which illuminates the wafer through the mask and knobs to change the positions of the wafer.

The wafer was aligned by moving the knobs which changes the wafer position along x and y coordinates while the z-coordinate knob was regulated to fix the focal plane of the image .The purpose of the alignment was to ensure that whole wafer covered up by the mask and we printed the structure in the middle of the substrate .

After the alignment, the wafer was subjected to expose under ultraviolet light. Before the exposing the parameters like exposing time, intensity of light and wavelength of the light was adjusted. For this procedure we used 365 nm wavelength, 15 seconds exposing time and the intensity of the light was about 12.5mW/cm$^2$.

After exposure, the wafer was taken out and placed on a horizontal plate so that reaction between photoresist and UV light could reach to stability

### 4.2.3 Developing

After the exposure, the next step was the developing of the structure which was just patterned on the wafer. Developing is a wet process where wafer was put into a wafer holder and immersed into a jar of a liquid chemical call developer. The wafer was agitated continuously into the solution and through observation we judged when the reaction was completed between the developer and resist .As the reaction proceeded the resist began to dissolve in developer and when the dissolution just finished we took wafer out the solution immediately . There was no fixed timing for the development process, and it varies with every individual fabrication process and the photoresist used. The correct time of 15 secs were found after several trials.

Positive resists are developed usually in a dilute alkaline solution. For that case MF-319 which had the pH 13 and the main component is Tetramethylammonium hydroxide and the overall concentration of the mixture is usually between 2-2.5 % concentrated as discussed in MICROPOSIT MF-319 DEVELOPER (DG), MSDS.

Then to stop the chemical reaction wafer was washed by the demineralize water and dried carefully with high pressure nitrogen so that it would not devastate or wipe out the structure which was just printed on the wafer

### 4.2.4 Inspection & measurements

After developing the structure was investigated thoroughly in the optical microscope to make certain that full structure was transferred correctly, and we didn't lose any pattern. After that the photoresist thickness was measured in different positions of the wafers by using Profilometer DEKTAK 150

## 4.3 First Etching for pillar Fabrication

After finishing the first photolithography the next task was the transformation of pattern on the substrate and fabrication of pillars with a height of 10µm. It was carried out in a dry etching machine Oxford Instruments 100 Estrella. This dry etching process utilized moderate level of RF power and pressure to combine both physical and chemical etching. The Boasch process was used in that case which uses a polymer to passivate the etching of the sidewalls. To make sure we etched with in range initially the process was carried out in small intervals and after getting satisfactory results it was performed in one cycle. The one whole cycle took about 20 minutes.

*Table 4-3represents the parameter used to etch 10-11micron to fabricate the pillars*

| | |
|---|---|
| ICP power (W) | 1750 |
| HF Power (W) | 70 |
| Total steps | 520 |
| Stage cycles | 45 |
| Total time (min) | 20 |

## 4.4 Stripping of positive resist

After completing the etching, we measured the height of the etched pattern with profilometer DEKTAK 150 which must be between 10-12µm. If the results were satisfactory then the next task was to prepare the wafer for second photolithography. For that purpose, the positive resist was stripped off at first with the solvents like acetone and isopropanol. The wafer was soaked in the beaker containing the solvents and placed inside the Ultrasonic bathtub. The wafer was dipped for 10 minutes for each solvent and then it was washed with demi water and dried with high pressure nitrogen gas. Usually there are some traces or strains remain on the surface which were removed by placing

the wafer in an Oxygen Plasma Cleaner for 10-15 minutes under vacuum and using the generator power of 300 W and oxygen pressure of 300sccm for 15 minutes. If there were still leftovers or stubborn stains the same procedure was repeated in plasma cleaner until we get the tidy and smooth surface of the substrate. The use of harsh chemical was intentionally avoided in this step as positive resist was relatively less complex solution.

## 4.5 Second photolithography with negative photoresist

After removing the resist, the next task was to pattern the 2nd layer on the structure by employing the photolithography method. This time the negative resist was incorporated and following steps were followed.

### 4.5.1 Coating

After making sure the wafer surface was cleaned during the stripping of the positive resist then it was coated with negative resist Az 125 nXT-10A in Spinner Sp100. To cover the whole area, it was spread with the dropper and made sure it covers the ¾ surface area of the wafer. The following coating programme was used in the tool.

*Table 4-4describe the specs of spinning program employed during photolithography with negative PR*

| | Speed | Timer | DC | AC |
|---|---|---|---|---|
| Step 1 | 0 | 0 | 0 | 0 |
| Step 2 | 900 rpm | 5 sec | 0 | 5 sec |
| Step 3 | 1000 rpm | 10 sec | 10 sec | 5 sec |

After coating with the resist, the wafer was placed on the metal plate for 5 minutes to provide settling time.

### 4.5.2 Soft baking

After depositing resist the wafers were soft baked so that the solvents present in the resist could evaporate and we get a better surface adhesion. For that purpose, the wafer

was placed on the hotplate for 60 minutes at a temperature of 130. This timing of the photoresist was very critical and special attention was made to avoid overheating.

### 4.5.3 Alignment and Exposure

The alignment and exposure step were done in mask aligner EVG620. This took a longer time than usual due to thick layer of resist and once it was ensured that all the alignment marks on the mask and wafers coincided with each other than the wafer was exposed under UV light for 600 seconds . After exposing wafers were placed on the metal plate so that the reaction between the resist and UV light could be stabilised

### 4.5.4 Developing

After exposing the wafer, it was dipped in the developer Az 326 MIF under constant agitation. It took about 400- 500 seconds to develop the resist but in some cases a portion of the pattern was developed and the other portion couldn't get developed even it was immersed in the solution for ten minutes with stirring .

### 4.5.5 Inspection

After developing the wafers were inspect in microscope and investigated the outcomes. For unsatisfied results the wafer were again developed and check.

## 4.6 Second etching for creating holes

Second photolithography was followed by the etching process which was again carried out in the dry etching machine Oxford Instruments 100 Estrella. The protective layer (photoresist) which was deposited during 2$^{nd}$ Photolithography identify the material to be removed and protect the material that is not to be etched. There were various strategies which were adopted during the second through hole etching. The ultimate target was to avoid the breaking of the wafers and preserve the structure.

For the first strategy the whole process was carried out in one long process which took almost an hour. The one big continuous operation was sub-divided into different cycles with different parameters.

### 4.6.1 First Recipe

After completing the operation most of the times the holes didn't appear, and the cavity depth was measured which was about 475-525-micron meter as measured by interferometer. There was still 50-100µm to get it through so the process was continued for the 2nd cycle.

*Table 4-5 represents the parameters used in long operation to create holes in the structure*

| | |
|---|---|
| Target(µm) | 575 |
| ICP power (W) | 2500-1750-3500 |
| HF Power (W ) | 90-100 |
| Total steps | 7205 |
| Total cycles | 600 |
| Total time (min) | 50 |

*Table 4-6 presents the parameters employed after one long operation to make holes*

| | |
|---|---|
| Target(µm) | 50-100 |
| ICP Power(W) | 2500-3000 |
| HF(W) | 100 |
| Total steps | 1600-1800 |
| Total cycle | 130-150 |
| Total time(min) | 10-15 |

After its completion we got the holes, but the boundary also become brittle and damaged. This method was tried with couple of wafers and both of times it didn't work.

### 4.6.2 Second Recipe

Then couple of more wafers were tested with modification in above parameters displayed in table 5.4 and it was tried to get the holes in one continuous operation .To achieve that the number of loops were increased and the following parameters were used

*Table 4-7 represents the parameters used to create holes in one long cycle*

| | |
|---|---|
| Targe(μm) | 575 |
| ICP power (W) | 2500-1750-3500 |
| HF Power (W) | 90-100 |
| Total steps | 9005 |
| Total cycles | 750 |
| Total time (min) | 60 |

This strategy also failed completely, and the wafers were damaged and even broken inside the chamber. The machine had to shut down and was cleaned and cooled manually for few hours.

### 4.6.3 Third Recipe

Then it was decided that procedure will be cut short into small steps instead of one continuous process to avoid extra heat generation and had more control on the etching process.

*First Step*

*Table 4-8 shows the first step parameters to create holes in the structure*

| | |
|---|---|
| ICP power | 3500 W |
| HF Power | 0 |
| Total cycycles | 400 |
| Total steps | 4101 |
| Total time | 28 minutes |

After finishing the first step we took the wafer out and let the machine cooled and cleaned by running the built in cleaning programme for 10-15 mins. Then it was continued with the following parameter

*Second Step*

*Table 4-9 shows the second step parameters to create holes in the structure*

| | |
|---|---|
| ICP Power | 3000 W |
| HF Power | 100 W |
| Total cycle | 350 |
| Total steps | 3323 |
| Total Time | 21 minutes |

After completion of this step the wafer was inspected, and the result was different for each case. In some cases, holes appeared in the structure and it was further investigated in microscope to observe the overall structure. In other cases where we didn't get holes the depth of the cavity was measured by using interferometry. Based on the measurement results the second step parameters were regulated with 90-150 cycles and got it through.

## 4.7 Anode Bonding

After the through hole etching the next task was to bond the silicon wafer with already fabricated glass wafers. The initial tests were carried out on plain silicon and the pyrex glass wafer. All the experiments were performed with wafer bonder EVG501 and 100mm diameter silicon wafers and glass wafer were used. Before proceeding to anode bonding process, the thickness of both wafers was measured to avoid any undue contamination on the surface of the wafer after cleaning procedure. There were certain steps which were performed during anodic bonding and are described below.

### 4.7.1 Wafer cleaning process

The first step in anode bonding was to clean the silicon wafer so precisely that it would become absolute free from all the contaminants which can distort the bonding process. To achieve this motive two cleaning strategies were endorsed initially, and details are given below.

*Recipe 1*

*Table 4-10 shows the cleaning recipe 1 employed to prepare the wafers for Anode Bonding*

| | |
|---|---|
| Stripper Solution | Piranha |
| Duration | 20min |
| Further cleaning | DI water and dry $N_2$ gas |

Here piranha is mixture of 2 parts of 98% $H_2SO_4$ and 1 part of 30% $H_2O_2$. To promote the cleaning process a buffered HF solution was introduced along with piranha in 2nd technique and the cleaning process was prolonged. The specs of 2nd recipe are detailed below

*Recipe 2:*

*Table 4-11 shows the cleaning recipe 2 employed to prepare the wafers for Anode Bonding*

| | |
|---|---|
| Stripper Solution | Piranha + HF |
| Duration | 30 minutes |
| Further Cleaning | DI water and dry $N_2$ gas |

The cleaning procedure was immediately followed by the anode bonding process

### 4.7.2 Align and Clamp Wafers

First, the clean Si wafer was placed on the bonding chuck with face up and after that clean Pyrex glass wafer was placed on top of the wafer with face down. Then these wafers were clamped together to hold the samples and field electrode were contacted with the bonding tool. Since these were plain wafers so the alignment was not problematic.

### 4.7.3 Getting started

The vacuum system was turned on which allowed evacuation down to $10^{-4}$ mbar. After checking all the warning lights and noted down the temperature reading , a recipe with modified parameters were selected to run .When the bonding process was finished ,

the system was vented and allowed the plate to cool. The bonds sample was taken out and inspected. Total time which were consumed on the experiment was about 4-5 hours.

These were the main parameters which were changed and play a key role in the bonding process. The trials were done for bonding Si and pyrex glass wafer and the details of the parameters are given below.

***Test 1***

*Table 4-12 shows the parameters of first test performed during si-pyrex anode bonding*

| | |
|---|---|
| Stack thickness | 4.6mm |
| Temperature | Top: 500°C; Bottom: 500°C |
| Force | 2500N |
| Voltage | 300V (12 min.); 500V (12 min.); 700V (12 min.) |

***Test 2***

*Table 4-13 shows the parameters of second test performed during si-pyrex anode bonding*

| | |
|---|---|
| Stack thickness | 4.3mm |
| Temperature | Top: 500°C; Bottom: 500°C |
| Force | 3000N |
| Voltage | 400V (12 min.); 600V (12 min.); 800V (12 min.) |

**Test 3**

*Table 4-14 shows the parameters of third test performed during si-pyrex anode bonding*

| | |
|---|---|
| Stack thickness | 4.1mm |
| Temperature | Top: 500°C; Bottom: 500°C |
| Force | 6000N |
| Voltage | 500V (15 min.); 700V (15 min.); 900V (15 min.) |

# 5 Results

## 5.1 First photolithography with positive resist

The experimental results for 1st photolithography with positive resist are discussed in the following sections.

### 5.1.1 Pattern transfer

Next to 1st photolithography with positive photoresist the wafers were inspected thoroughly using microscope to check, whether pattern is transferred correctly or not. The inspection was accomplished using Leica Optical Microscope of different magnifications to identify any defect properly. The result evaluation will decide the process would be continue with same wafer or not. The most crucial issue which was regarded during inspection included alignment, line width, and transformation of the pattern.

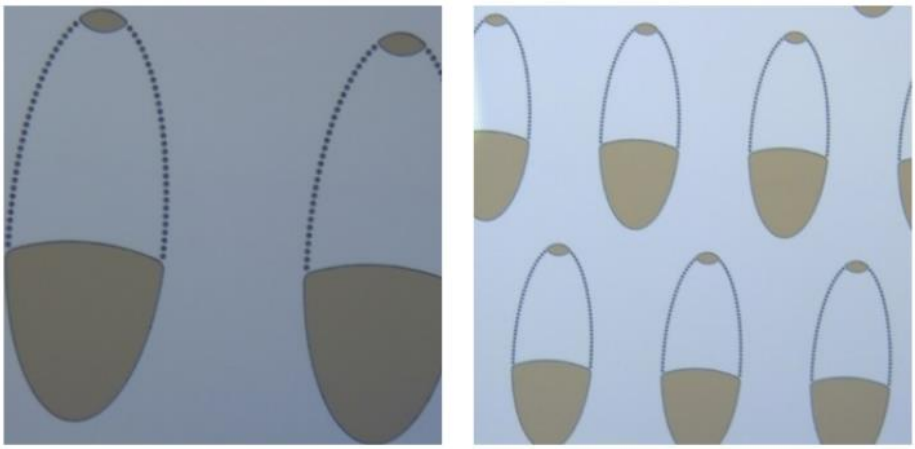

*Figure 5-1 displays the pillar structure arranged on the elliptical profile. The image was taken by Leica Optical microscope with bright field mode and 5X, 10 X magnification*

Figure 5-1 shows that the pattern was transferred successfully without any defect. The same pattern imprinting was got on the complete wafer. This kind of structure was obtained after several trials and the entire wafer structure was found to be very smooth.

Figure 5-2 image gives more closer look to get understanding of the structure which was supposed to be achieved after the first photolithography with positive photoresist.

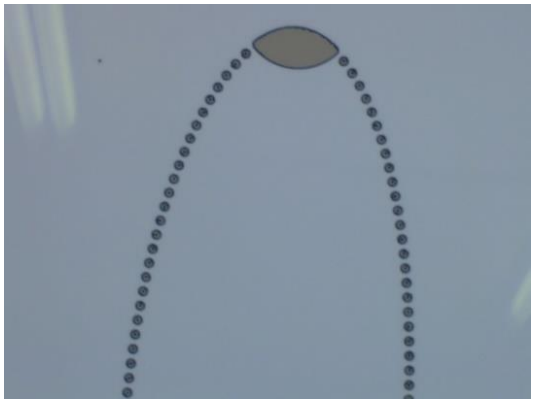

*Figure 5-2 was taken with Lecia Optical microscope in bright field mode and 20X magnification which shows the pattern of pillars arrangements*

Figure *5-2* demonstrate the well-magnified image of the pillar structure and the space between the pillars can be visualized. The line width is also perfect which was critical for the process.

### 5.1.2 Resist Thickness

Obtained results make it possible to use wafer for further procedures. The thickness of the photoresist was measured and appropriate range for the thickness was between 10 µm to 25 µm. The information of thickness helped in subsequent etching process.
The measurement was taken after completing the 1$^{st}$ lithography with profilometer to measure the thickness of the positive photoresist as shown in Figure 5-4 .The thickness of the photoresist was measured in three different position of the wafer and results were found almost the same. Parameters used to measure the thickness of photoresist are tabulated in Table 5.1.

*Table 5-1 displays the parameters used for measuring the thickness of photoresist*

| | |
|---|---|
| Type photoresist | +1813 |
| Taken | After developing the photoresist |
| Profile Used | Hills and valleys |
| Thickness measured | 2.2µm |
| Scanned length | 2000µm |
| Measurement range | 65.5µm |

| | |
|---|---|
| Time | 35 sec |

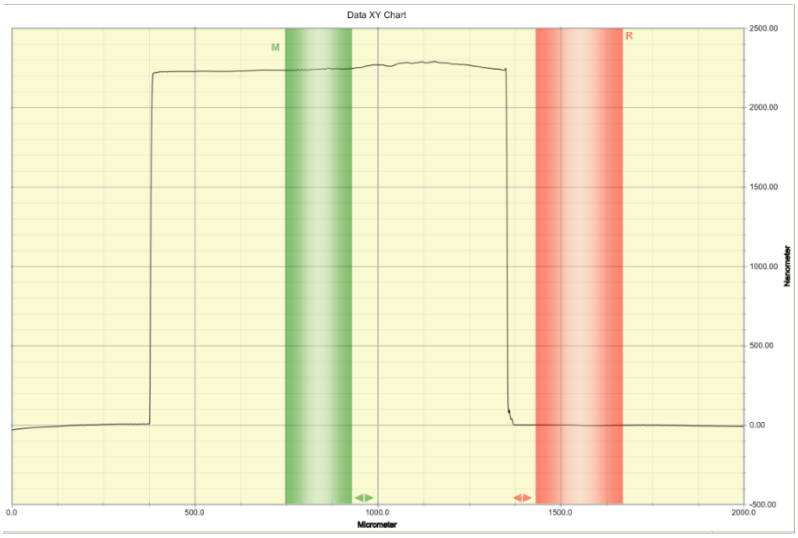


*Figure 5-3 was taken by Profilometer DEKTAK 150 to measure the thickness of photo resist after developing photo resist, the x-axis is the length of scanned area in[µm], y-axis is the information about height or thickness of photoresist in [µm]*

Figure 5-3 shows that the height of the photoresist is within specified range of 2.2 µm. Another example of measurement for positive photoresist thickness is described under.

*Table 5-1 shows the parameters used to measure the thickness of the photoresist*

| | |
|---|---|
| Type photoresist | +1813 |
| Taken | After developing the photoresist |
| Profile Used | Hills Valleys |
| Measured Thickness | 1,6µm |
| Scanned length | 1500µm |
| Scanning Time | 45sec |

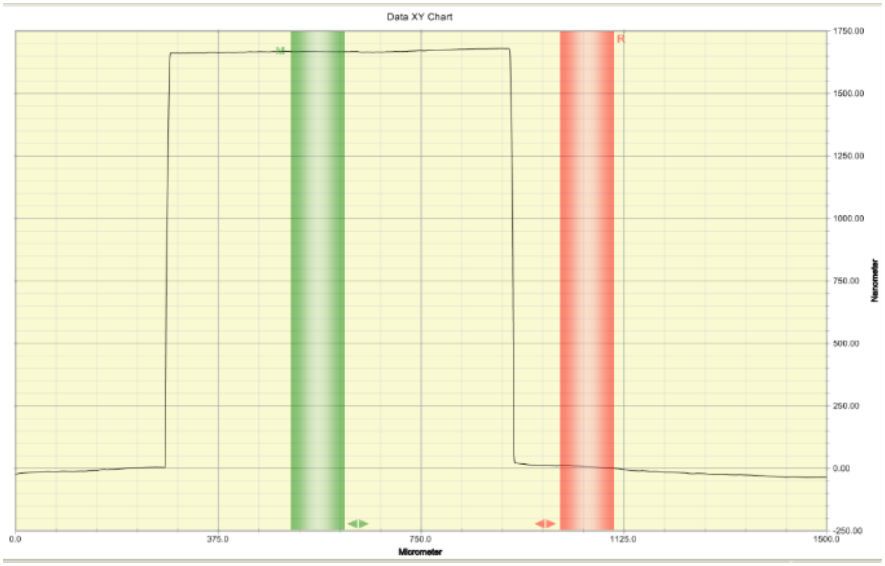


*Figure 5-4 Thickness measurement has been performed by Profilometer DEKTAK 150 to measure the thickness of photo resist after developing positive photoresist, the x-axis is the length of scanned area in[µm], y-axis shows information about height of photoresist film in [µm]*

Figure 5-4 shows the measurement which was performed to evaluate the resist thickness. The measured thickness of 1.6 µm was within the recommended range

### 5.1.3 Challenges during first photolithography

The accuracy and precision of result was complicated. Figure 5-5 exemplifies inferior results obtained during 1st photolithography.

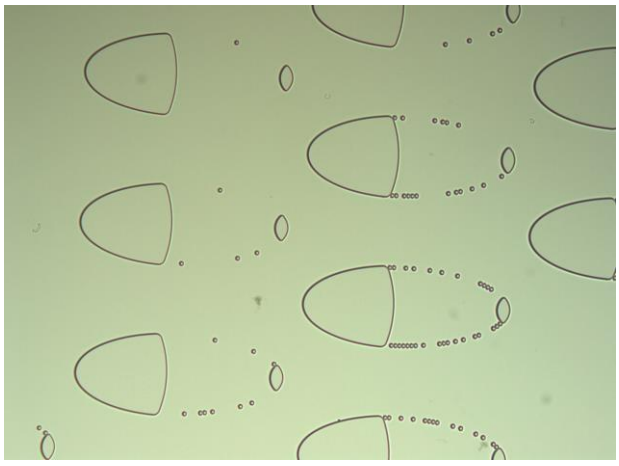

*Figure 5-5 Lecia Optical microscope in bright field mode and 5X magnification which shows the missing pattern of pillars arrangements*

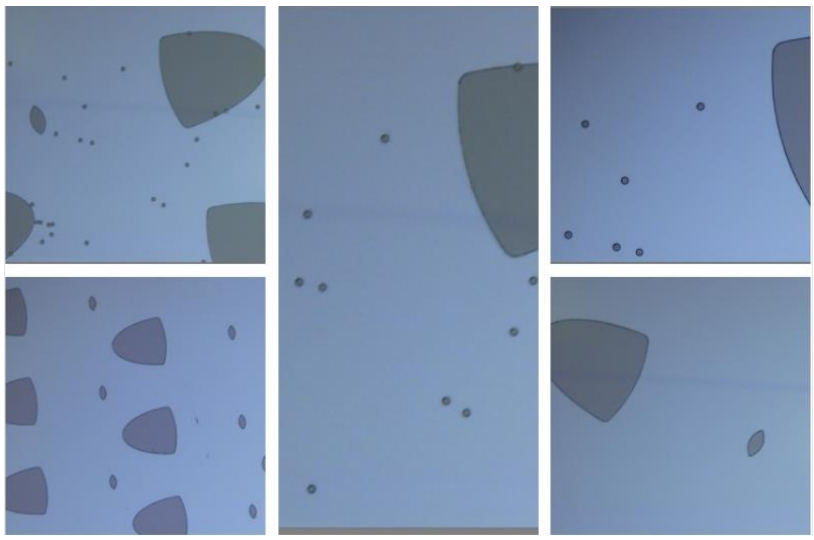

*Figure 5-6 Missing structures during first lithography. these figures were taken with Lecia Optical microscope in bright field mode and in different magnifications.*

Figure 5-5 6and 5-6 illustrates the inaccurate and defective results which were acquired during first lithography. Sometime, such pattern was found all over the substrate or within some specific place in the structure.

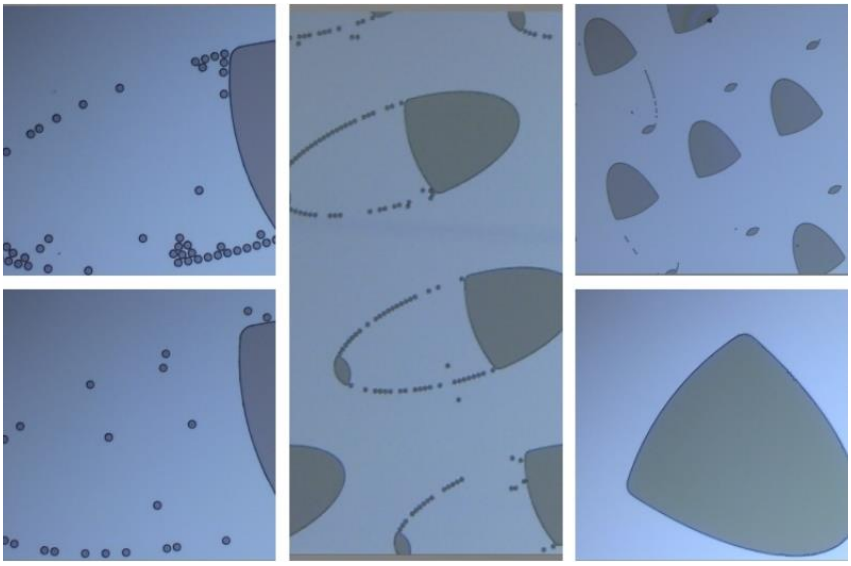

*Figure 5-7 Missing structures during 1st lithography. these figures were taken with Lecia Optical microscope in bright field mode and in different magnifications*

Figure 5-7 represents the classic examples of the impaired structure which were obtained frequently during the trials with 1st lithography. It can be recognized that high aspect ratio of the structure presented a huge challenge to define the right protocols for the exposure

and developing time. There were mixed results in different part of the structure; in some cases, pattern was lost partially and in some cases it was lost completely.

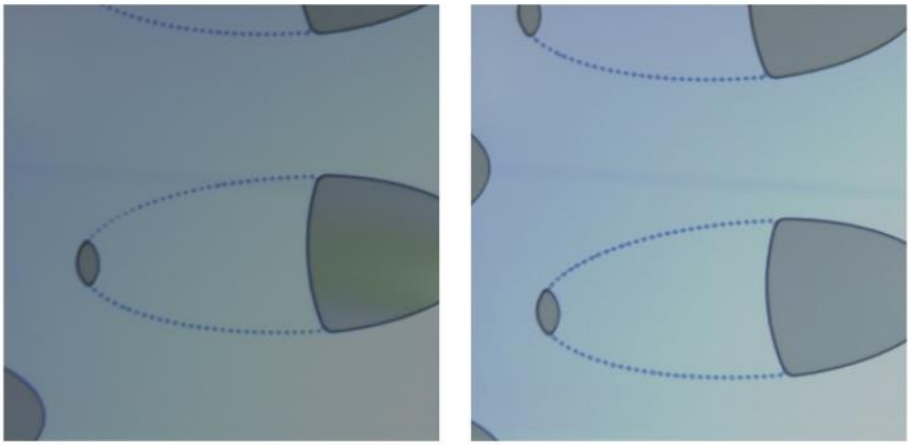

*Figure 5-8 Line width of the structure during first photolithography. The image was taken with Leica mobile in different magnification*

Figure 5-8 shows degraded line width obtained during 1st lithography. The most probable cause of this could be over developing and under exposure.

## 5.2 First Etching

Next to having an accurate result from 1st photolithography, the pattern was transferred to the silicon substrate through subtractive technique etching. The process required that the pillar height should be about 10 µm - 12 µm which means we needed to etch >10µm resist thickness which was achieved through Boash Etching Process. To control the process and ensure we did not exceed the required depth it the etching was being performed in small intervals and after the protocols were set up then it was carried out in single step. Following figure is an example when the etching was performed in short interval to measure how deep we had etched already.

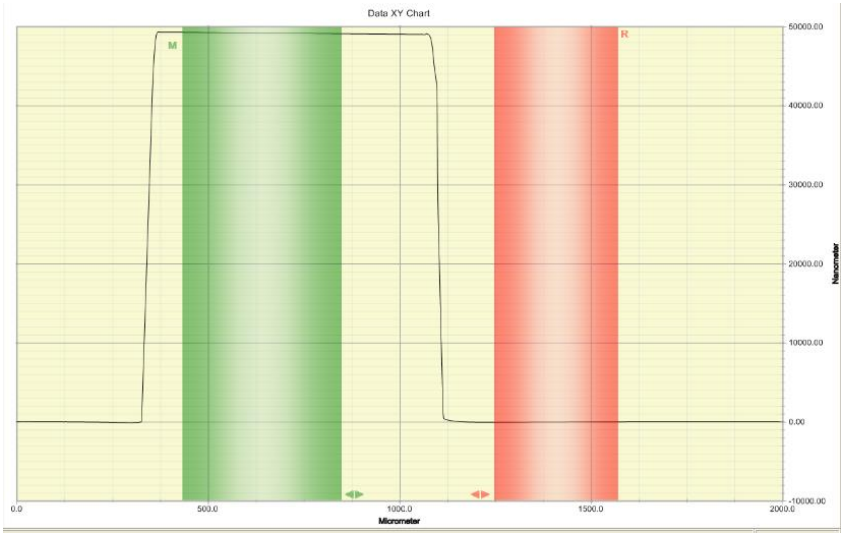


*Figure 5-9 Measurement performed by* Profilometer DEKTAK 150 *to measure the height of fabricated pillar with respect to silicon substrate after small interval of dry etching process ,the x-axis is the length of scanned area in[µm], y-axis shows information about height of pillars [µm]*

*Table 5-2 shows the parameters used to measure the height of the fabricated pillars during dry etching process*

| | |
|---|---|
| Type photoresist mask | + 1813 |
| Taken | During first etching for pillar fabrication |
| Profile Used | Hills and Valleys |
| Measured Depth | 4.9 µm |
| Scanned length | 2000 µm |
| Scanning Time | 35 sec |

Figure 5-9 indicates that we had etched almost halfway of the required height and process could be continued in the same strategy

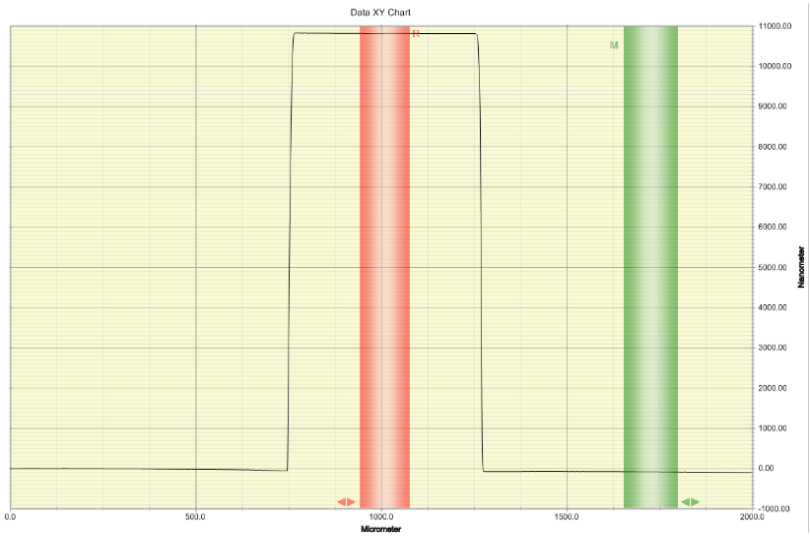


*Figure 5-10 Measurement has been performed by* Profilometer DEKTAK 150 *to measure the height of fabricated pillar with respect to silicon substrate after dry etching process, the x-axis is the length of scanned area in[µm], y-axis shows information about height of pillars [µm]*

*Table 5-3 shows the parameters used to measure the height of the pillors*

| | |
|---|---|
| Type photoresist mask | + 1813 |
| Taken | After first etching for pillar fabrication |
| Profile Used | Hills and Valleys |
| Measured Depth | 10.89 µm |
| Scanned length | 2000µm |
| Scanning Time | 30sec |

The figure 5-10 exhibit the total required height of the pillars which was achieved by performing dry etching process in two short intervals.

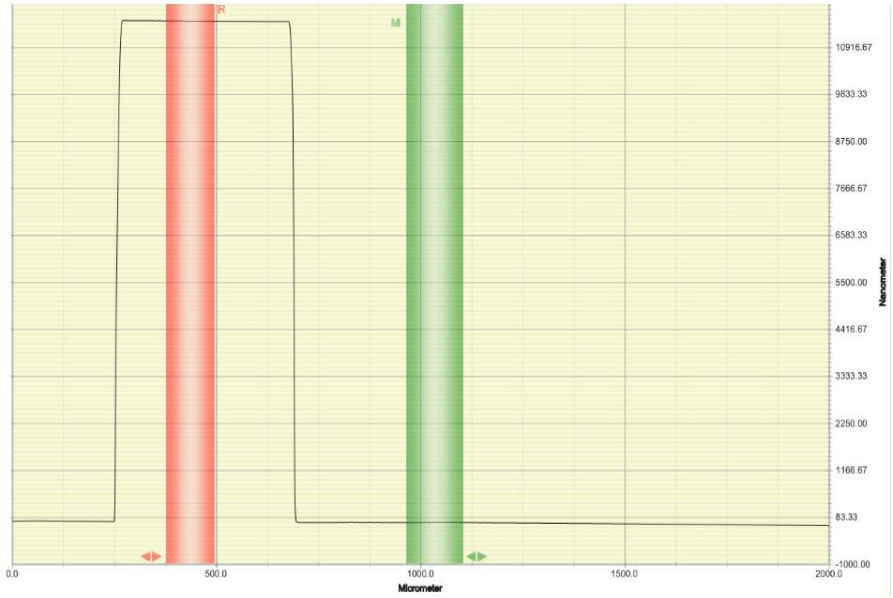


*Figure 5-11 Measurement has been performed by* Profilometer DEKTAK 150 *to measure the height of fabricated pillars with respect to silicon substrate after dry etching process , the x-axis is the length of scanned area in[µm], y-axis shows information about height of pillars [µm]*

*Table 5-4 shows the parameters used to measure the height of the pillors*

| | |
|---|---|
| Type photoresist mask | + 1813 |
| Taken | After first etching for pillar fabrication |
| Profile Used | Hills and Valleys |
| Measured Depth | 11.55 µm |
| Scanned length | 2000µm |
| Scanning Time | 30sec |

Figure 5-11 shows that the measurement taken by profilometer on silicon wafer in order to fabricate the pillars next to the Borsch etching process in one cycle. Since it was not very deep so profilometer was appropriate tool to measure the height of etched material.

## 5.3 2nd photolithography with negative Photoresist

### 5.3.1 Pattern Transfer

After removing the positive resist, the wafers were exposed for hole integration in the structure. Once the photoresist was developed then the structure was viewed in the microscope to find out the accuracy of the procedure. Here also the main issues which were highly concerned were alignment, line width and transferred pattern.

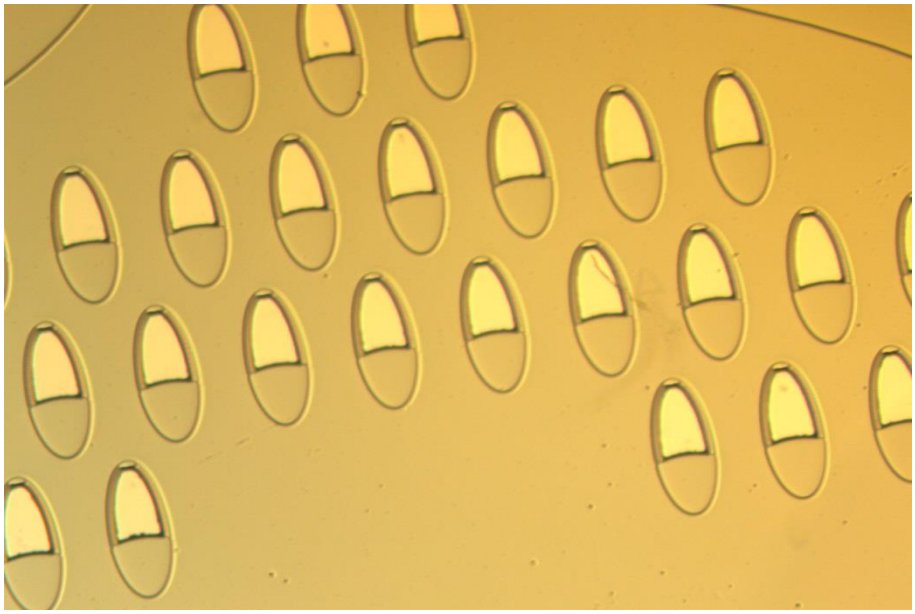

*Figure 5-12 shows the pillar structure and substrate surface between the pillars to illustrate the design of device. The image was taken by Leica Optical microscope with bright field mode and 2.5X magnification*

Figure 5.12 gives nice details about the arrangements of pillars structure patterned on the silicon wafer although the fine details are not visible but still it can be assessed the elliptical separation unit profile and the results which we got after successfully aligning the holes pattern inside the pillars.

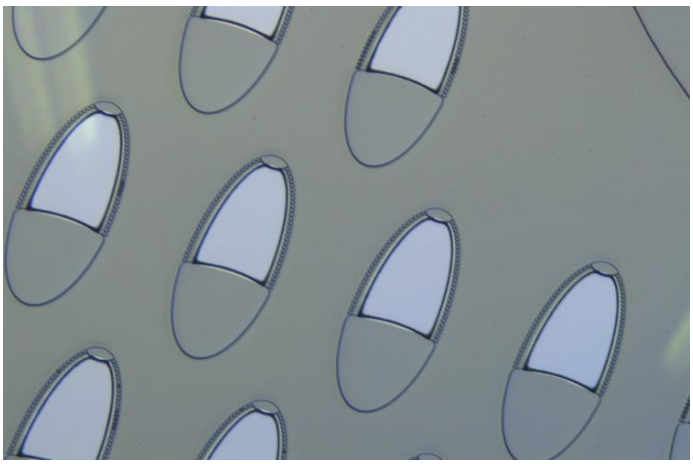

*Figure 5-13 shows the pillar structure and substrate structure between the pillars. The image was taken by Leica Optical microscope with bright field mode and 5X magnification*

Figure 5-13 expresses new pattern that was successfully aligned with the previous layer. The gap between the new structure and pillars is also visible which is critical for through hole etching. Inside the pillars wall the silicon substrate is visible where we desire to have holes by through hole etching.

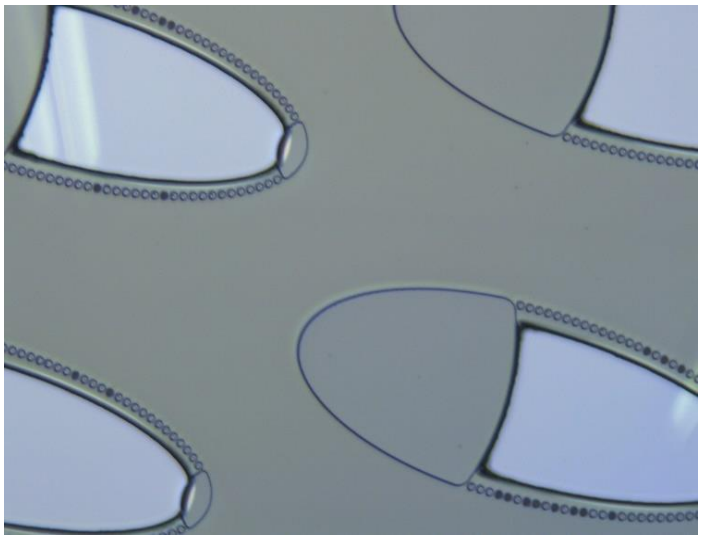

*Figure 5-14 shows the pillar structure and substrate surface between the pillars. The image was taken by Leica Optical microscope with bright field mode and 10X magnification.*

Figure 5-14 gives more clear view of the design and purpose of the 2nd mask which was aligned very carefully with the first layer and the clean removal of photoresist after developing can also be observed.

### 5.3.2 Thickness of the negative photoresist

If there was nothing wrong with the wafer after 2nd lithography, then it was preceded with the through hole etching but prior to that it was important to measure the resist thickness to estimate the full depth which must be more than 40µm.

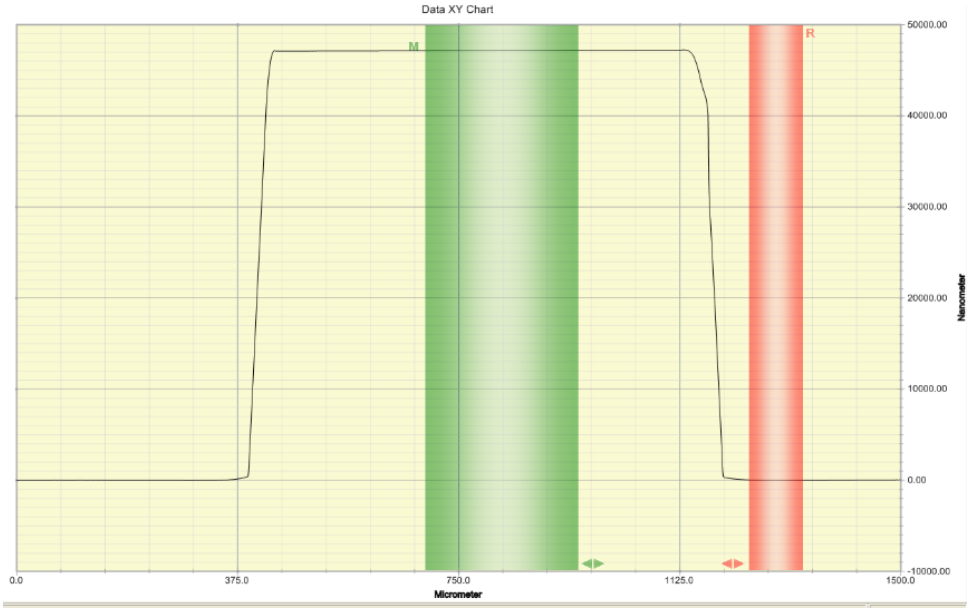


*Figure 5-15 display thickness measurement has been performed by profilometer to measure the thickness of film after developing negative photoresist, the x-axis is the length of scanned area in[µm], y-axis shows information about height of photoresist film in [µm]*

*Table 5-5 presents the parameter used to measure the height of negative resist after developing*

| Type photoresist | Negative |
|---|---|
| Taken | After developing with negative resist |
| Profile Used | Hills and Valley |
| Measured Depth | 47.17 µm |
| Scanned length | 1500 µm |
| Scanning Time | 35 sec |

Figure 5-15 featured the measurement which was performed to evaluate the resist thickness. The measured thickness of 47.17 µm was within the recommended range

### 5.3.3 Challenges with 2nd Lithography

*Figure 5-16 shows that after 2nd photolithography the structure was not fully developed. The image was taken by Leica Microscope in bright field mode and 2.5X magnification.*

The above figure 5-16 was taken after the second lithography and shows the pattern was not fully transferred. The black marks are the photoresist which supposed to be fully removed after the developing. These black marks were the spots where holes didn't appear when it was tried to etch.

## 5.4 Through hole etching

Next to 2nd lithography the wafer was supposed to undergo through hole etching . The wafer thickness was about 525µm in addition to resist thickness which was about 50 microns. This etching was executed in short intervals to avoid the structure damage. The depth of cavity which appeared after two cycles of etching was measured by Interferometer Wyke NT9100.

Figure 5-17 was taken after 36 minutes of deep etching which was performed in short intervals of 18 minutes each. The measurement was performed using VSI mode and the measured depth is 362µm in some part of the structure. It is more than the half to get the through hole in the structure. Different color shows the etching progress in different regions of the structure. From the figure, it is obvious that etching has not destroyed the structure and the process proceeds in a smooth and desired way.

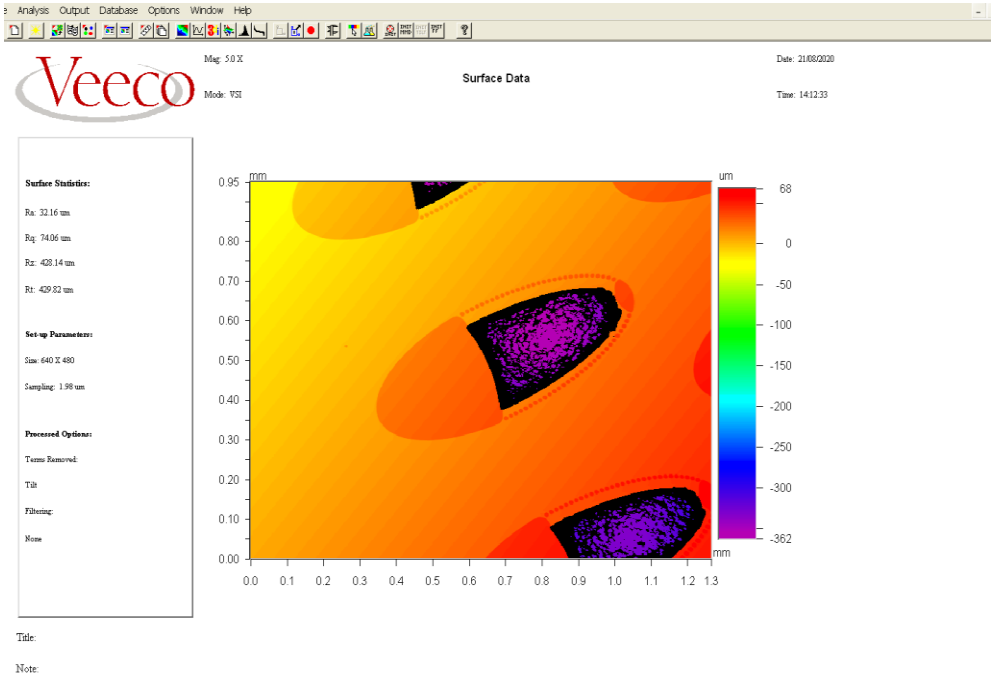


*Figure 5-17 measurement done by Interferometer Wyke NT9100. to determine the etch depth, along the x-axis the total area of interest and along y-axis its depth of the valley.*

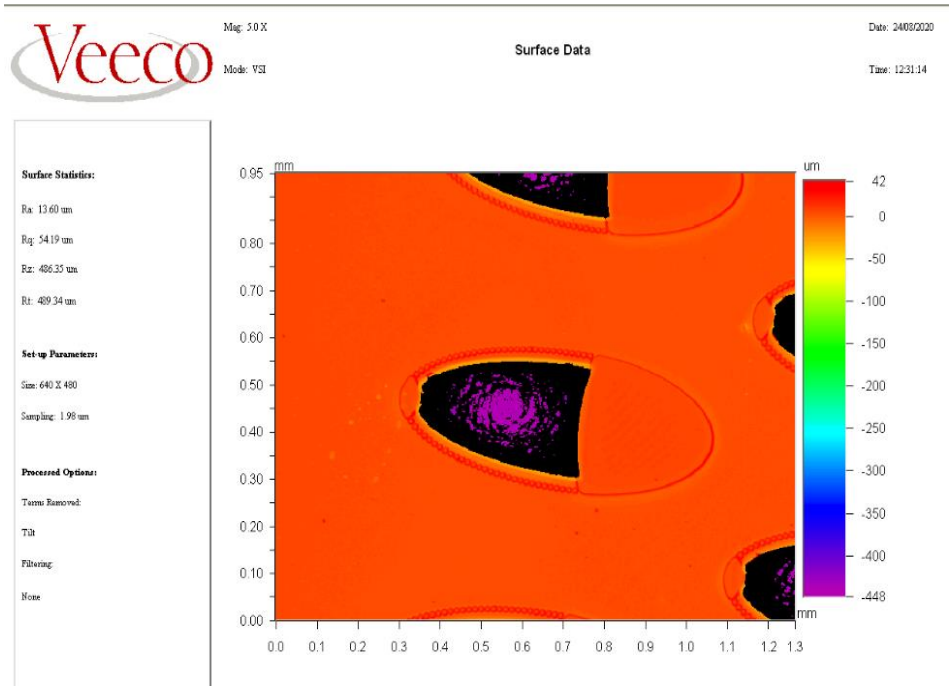


*Figure 5-18measurement done by Interferometer Wyko NT9100. to determine the etch depth. along the x-axis the total area of interest and along y-axis its depth of the valley.*

Figure 5-18 shows the measurement that was done after 60min of deep silicon etching and was taken in VSI mode. The measured depth is 448µm and it is not through, yet which

means at least 100µm depth remained to create a hole. In this figure there is no uneven depths in the substrate and the pillar structure is still intact and etching had not widespread from its boundary. in some cases, the wafer got the holes in some places and still it was not through in other places and we had to etched more which most of the time resulted in over etching in some regions.

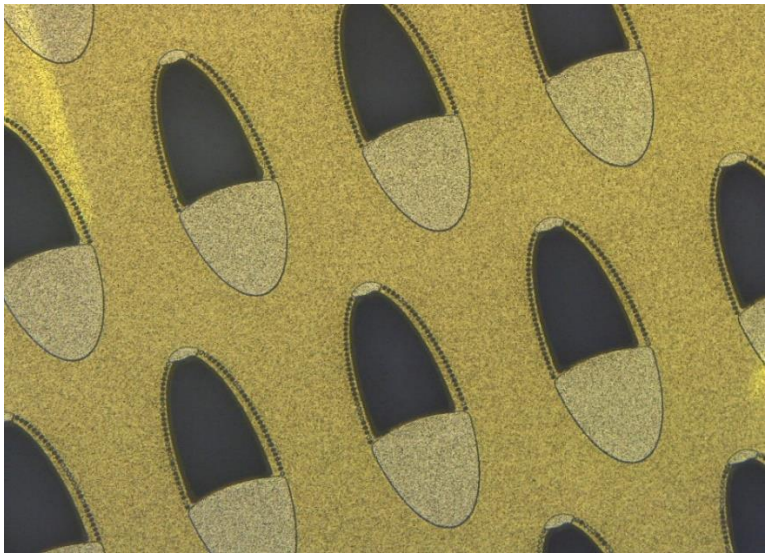

*Figure 5-19 structure of the wafer was observed after the deep hole etching through Leica optical microscope. the mode is Bright field, and magnification is 5X.*

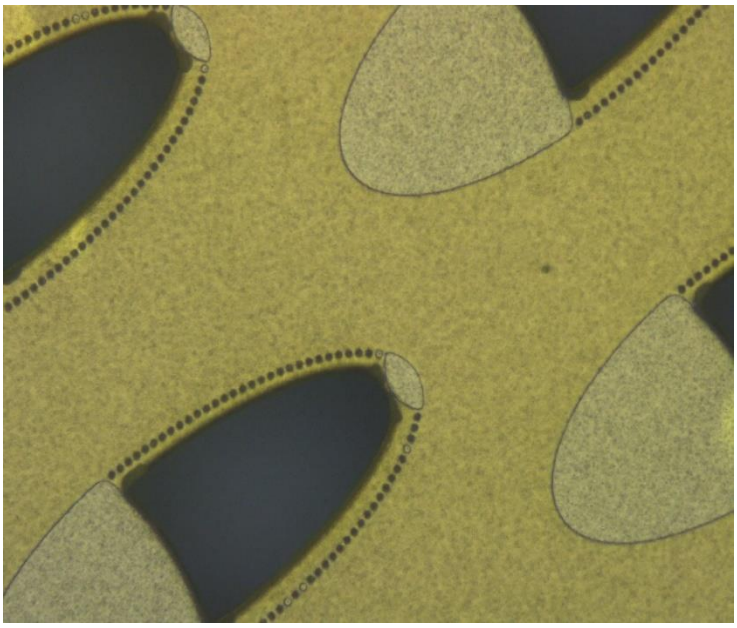

*Figure 5-20structure of the wafer was observed after deep through hole etching .The image was taken by using bright field mode and 10X magnification.*

Figures 5-19 and 5-20 were taken after the holes appeared in the wafer to investigate the etching process. The images show perfect and successful results, the black part in the

structure shows the holes and can be observed that it is not over-etched, and pillars are still distinct and visible. there is still a space between pillars and holes which was highly recommended for the process. In addition, the etching did not damage the structure which led to establish the selection of etching parameters and strategy.

### 5.4.1 Challenges in Through hole Etching

During the through hole etching it happened many times that the etching process destroyed hole structure. or even broke the wafer in pieces or from the corners sometimes.

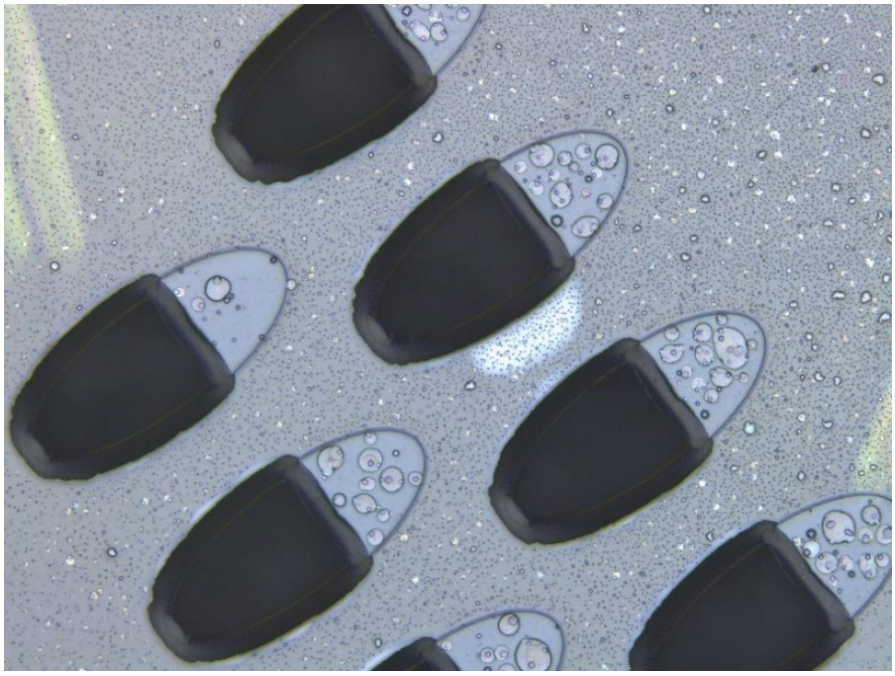

*Figure 5-21 the structure of the wafer was observed after the deep dry silicon etching . The image was taken by Leica microscope in bright field mode and 5X magnification*

Figure 5-21 revealed the disastrous progress in through hole etching and it can be seen that even though the holes are appeared, but they have crossed the desired space and damaged the whole structure. Majority of the results were obtained like this.

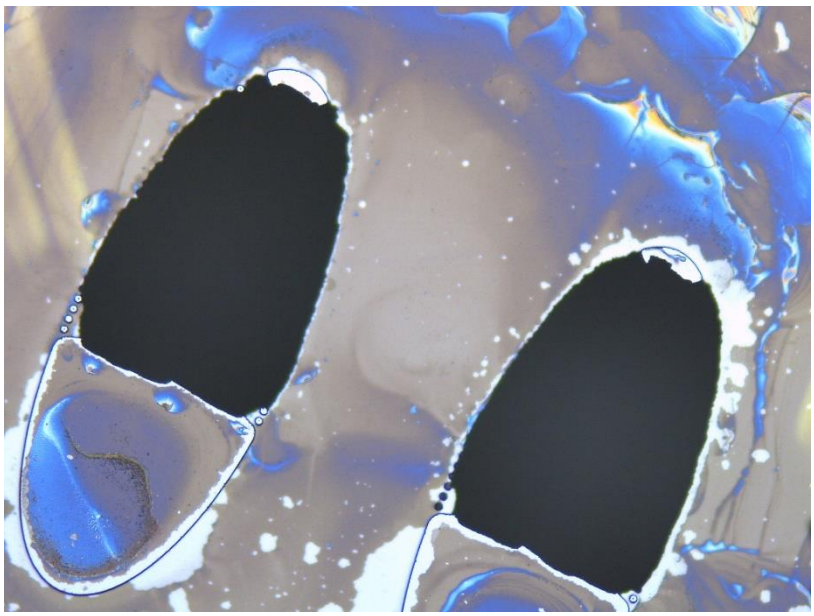

*Figure 5-22 the structure of the wafer was observed after the deep dry silicon etching. The image was taken by Leica microscope in bright field mode and 10X magnification*

Figure 5-22 further depict the unsatisfactory through hole etching in which etching had gone over its limits. The etching also damaged the aligned pillars. These kinds of results attained due to non-uniform etching process where we got both under etch and over etch places on the same wafer since it was very uncertain and unpredictable process. It took a while to get control over the etching progress.

## 5.5 Anode Bonding

The anode bonding was performed after the completion of the fabrication of processing layer, it was supposed to bond 3 wafer stack where top and bottom layer will be composed of glass. The initial trials were carried out with different strategies and the outcomes are shared below.

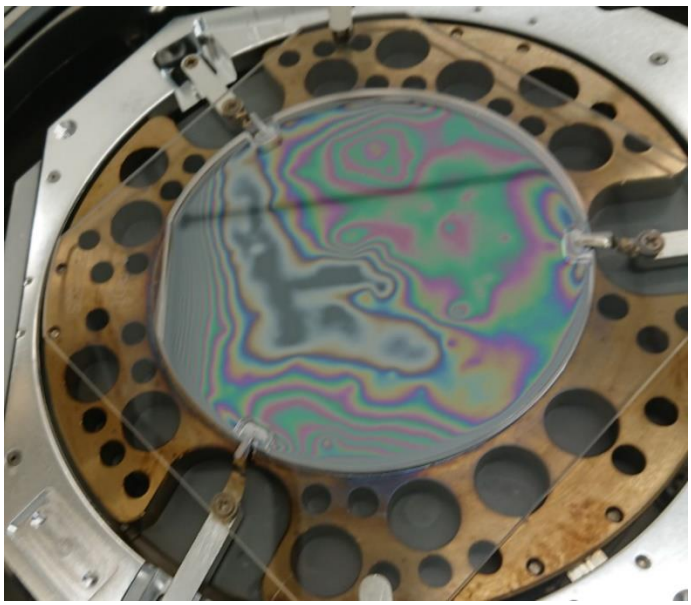

*Figure 5-23 Si-Pyrex bonding - 500°C; 2500N; 300V/500V/700V*

Figure 5-23 was taken after completion of test 1 during trials of anode bonding. The stake thickness was 4.6mm and the result was deficient as it was not tightly bonded. The bonded area was approximately less than 30% and had some voids caused due to particles trapped inside.

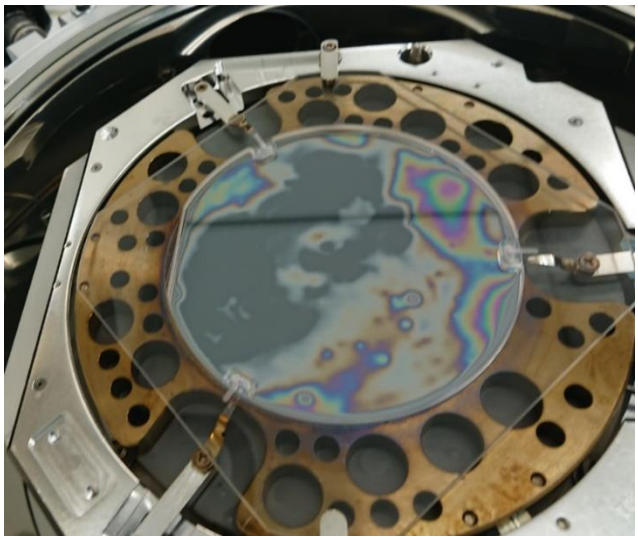

*Figure 5-24 Si-Pyrex bonding - 500°C; 3000N; 400V/600V/800V*

Figure 5-24 was taken after the 2nd trial during anode bonding and thickness of the stack was 4.3mm. The result was not up to the mark as there was still gap between two wafers. The bonded area was approximately more than 30% and contained voids caused by the impurities and contaminations present on the substrate surfaces.

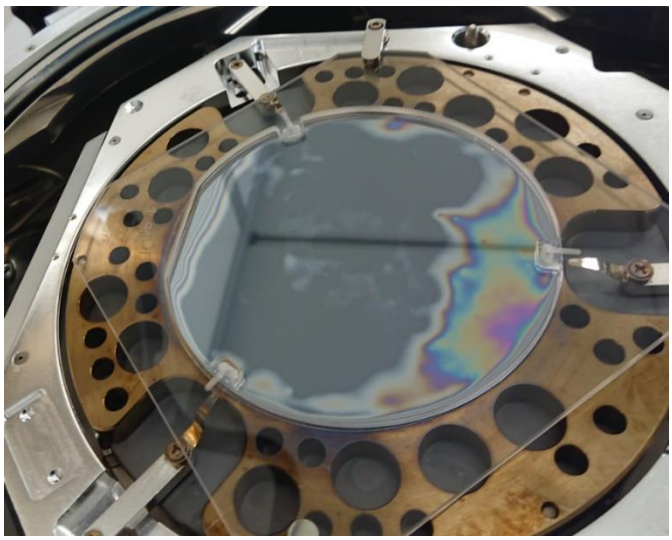

*Figure 5-25 Si-Pyrex bonding - 500°C; 6000N; 500V/700V/900V.*

Figure 5-25 portrays the result obtained after performing test 3 where the total thickness of the stack was 4.1mm. it was the relative best result which was got so far. Two wafers made a compact interface and exhibited no looseness and gaps in bonded area. Total bonded area was marked between 60-70%

# 6 Discussion

The PASECOR is an emerging technology which deals with particles separation and microfluidics affairs. The idea and concept of the technology has been demonstrated in papers and creating prototypes since last few years. These prototypes were mostly patterned on plastic by 3d printing or injection moulding. But due to limitations of feature sizes in these techniques it was highly desirable to promote to the next level. In the novel project the focus was how to fabricate the silicon chip such that we could be able to separate the micro particles in approximately 5micron range and make a feasibility for oil industry where they were concerned with oil eating bacteria . To prove that point the device will be tested for micro algae (2-10 micron) . The fabrication on silicon was very sensitive and complicated task. There were different layers and various process and stages involved in the whole process. Every step took many trials before we could proceed to the next level but the most crunching part was after successfully moving on the next level and if there something went wrong during next level then that led to start from the level one again .In all of these trials it took hard and long efforts along with time and resources of the lab.

More than 30 wafers were tested to achieve the target of 5 wafers with correct pattern, for the last finishing process i.e. anode bonding .The current epidemic had the adverse effect on the whole procedure and made the progress very slow and even halted from march to July as the clean room was closed due to lock down and then summer vacations and after the lock down there were strict protocols about the presence of people in the lab and there was long waiting time to book the equipment. Besides all that it was managed to make a significant progress and most importantly we have developed protocols for the future practices. I have discussed major challenges some were faced during the whole process.

## 6.1 First photolithography with positive resist

### 6.1.1 Cleaning strategies

Cleaning techniques were employed to ensure that substrates were completely free of contamination and foreign particles as it endured the fabrication process .In case there were any left overs on the surface , they distorted or damaged the current as well as the subsequent processes. On the other hand, a well clean and uncluttered surface promoted the adhesion of the photoresist with substrate surface and improved the quality of the results. There could be many sources of contaminants present on the wafer surface which included solvent stains, smoke particles and dust from environment and equipment. These undesired foreign matters or defects caused by these could be observed more accurately by using microscopic techniques. To encounter these imperfections there were various techniques which were adopted to clean the wafer surface including both wet and dry methods. Wet methods incorporated the solvents like acetone and Isopropanol to remove these pollutants. Acetone has the property of dissolving organic impurities and isopropanol is suitable for expelling non-organic substances and residuals of acetone from substrate surface. To escalate the cleaning process an ultrasonic agitator bath was used in which agitation , caused by the ultrasonic energy , detached or loosened the debris from the wafer which were washed away completely by demineralized water and lastly blow dried with nitrogen gas. During the photolithography process it was observed that some wafer did not yield the desired results so the cleaning process was more emphasized and after washing with chemical reagents a dry cleaning method was employed where wafers were placed inside the Plasma cleaner Alpha Plasma AL 18_ for extra cleaning to assure that substrate did not contain any kind of pollutants , unpleasant substances and stains.

### 6.1.2 Positive resist selection

For the first photolithography a positive photoresist was applied on silicon wafer which means the exposed region under ultra violet (UV) light become more soluble and during developing the exposed photoresist dissolves and unexposed resist remains on the surface of the wafer. The selection of the photoresist was critical and for that procedure PR S-1813 was preferred as it permit patterning of excessively high aspect ratio

structures, with relatively low exposure dose as stated in material datasheet MICROPOSIT™ S1813™ G2 POSITIVE PHOTORESIST.

### 6.1.3 Spin coating and importance of photoresist thickness

The thickness of the deposited film played a vital role in the ongoing and consecutive steps. There were three key factors which could had an impact on the thickness of the layer which are

- viscosity of PR
- spin speed
- time

Among those the combination of time span and spin speed selected for each step defined the total thickness of the deposited layer. During spin coating the centrifugal forces caused the resist to spread towards the edges of the wafers where it developed and evolved continuously until expelled on the inner wall of the spinner. The empirical expression for thickness T is given by [26].

$$T = \frac{kc^{\beta}\eta^{\gamma}}{\omega^{\alpha}}$$

where K = the overall calibration constant

C = the polymer concentration in g/100ml solution

Ƞ=intrinsic viscosity

$\boldsymbol{\omega}$= the number of the rotation per minute and $\alpha$, $\beta$ and $\gamma$ are various exponential factors

Thickness of resist defines whether the deposited layer can behave as reflective or antireflective film during exposure with UV light .It was observed that when the thickness was precise the film acted as anti-reflective material and most of the ultraviolet light during the exposure was absorbed and we got correct geometry on the wafer , otherwise for more thick film it acted as reflective layer and less light was absorbed and in the end we missed the important pillars of the structure. This kind of problem is visible in figure 5-6 and 5-7 where the main structure of the pillars was absent from the structure.

Normally a chemical called the primer is used which provides an intermediate layer, most commonly HMDS( Hexamethyldisilane provides the intermediate hydrophobic surface

which promotes the adhesion of photoresist but it was avoided due to sensitivity of structure and selected photoresist.

### 6.1.4 Significance of Soft bake

After depositing the resist on the wafer surface, it was heated for short span of a time in order to remove the residual solvents and photoresist stresses it might contain. A smooth and clean surface of the hot plate was required to ensure uniform heating and good thermal contact. Timing and the temperature were the critical for the accuracy of the process as excessive heating could damage the polymer structure of the polymer and photoactive compound present in the resist and its senility is also affected by the heating. The soft bake usually reduces the photoresist thickness up to 15 to 20 %.

### 6.1.5 Challenges regarding aligning and exposing

In this process the UV light provided the intensity, special characteristics, uniformity, and directionality to the features of the structure and the whole substrate surface. UV light triggered a chemical reaction between photoresist and the light as a result the impression on the masks were printed on the film of photoresist. During trials different exposure times were investigated. Generally, for the positive photoresist with thin layer the short exposing time was obligated and for that reason different trials were carried out by setting the exposure time between 10-25 sec. During exposure there could be two possibilities whether the substrate is over-exposed or under exposed. Overexposing contributed to turn the geometry profile into reverse direction and on the other hand underexposure caused slop in geometry. The best results were achieved when the wafers were exposed for 15 seconds and it defined the limit of under exposure and over exposure. During some initial trials after exposure the wafer was also post baked for 2 minutes at 115 $C^o$ then it was skipped due to unsatisfying results as it was suspected that high temperature caused the resit to flow and it damaged the pattern.

For the first photolithography the alignment was not a critical issue as just needed to make sure that whole silicon wafer boundaries matches with the glass mask edge and we got the pattern in middle of the wafer so that it could be aligned perfectly for $2^{nd}$ layer as well as it was important in anode bonding . After few trials, the actual technique of the alignment was learned.

### 6.1.6 Challenges regarding first developing

Developing is a wet process in which wafers are immersed physically in the solution and initiate a chemical reaction between resist and the developer. The development results are better if stirring is applied continuously to speed up the development process. Positive resists are developed usually in a dilute alkaline solution. For that case MF-319 which has the pH 13, Tetramethylammonium hydroxide as main component and the overall concentration of the mixture is usually between 2-2.5 % concentrated as discussed in material data sheet MICROPOSIT MF-319 DEVELOPER (DG).

The timing of developing process is always integral and crucial for developing process. Like exposure there were again two possibilities during developing which were under developing and overdeveloping. If the time interval exceeded than the optimal specific time interval then it caused overdeveloped resist and we lost the important details of the structure otherwise developing for short span of time led to under develop structure and it left too much resist on the surface of the wafer . Both reactions affected the line width of the structure negatively. The geometry of the structure had high aspect ratio which created difficulties during developing, and there was a thin line between overdeveloped or underdeveloped resist. Overdeveloping of resist is irreversible and it made the resist to swallow and broke the structure. An example of overdevelop resist can be marked in figure 5-6 and 5-7. Underdeveloped resist prevented the access to minute features and left excessive resist on the substrate surface. To overcome these issues the developing time was increased in short interval to find optimal developing time.

So, during trials many different timings were tested between 10-25 seconds and after many attempts the correct timing was determined between 12-15 seconds. Then to stop the chemical reaction wafer was washed by the demineralise water and dried carefully with high pressure nitrogen so that it would not demolish the structure just printed on the wafer.

About 1-1.15 litre of developer chemical was enough to develop 5-7 wafers for positive resist - After crossing that limit the developer took longer time and lost its strength and became non-reactive so was replaced with fresh solution

### 6.1.7 Inspection techniques

Then the structure was observed thoroughly in the optical microscope to make certain that structure was transferred fully and correctly. Mostly the wafers were investigated for following issues

- Alignment
- Defects
- Line width

It was monitored closely that wafers were perfectly aligned with mask and structure was acquired at the middle of the wafer along with the pattern image was in good focus .Line widths or critical dimensions were measured using the microscope and it was ensure that dimensions were into the desired specifications . The frequent issue was the defects in the transferred pattern, peeling of the resist and scumming (problem related with developing time). After thoroughly inspection thickness of the photoresist was measured by using the profilometer before subsequent etching process.

## 6.2 Pillar Etching and Stripping of photoresist

After inspecting the wafer, we proceeded with pillar etching in which pillars height of 10 micron was achieved. This process did not cause any major trouble and went quite smoothly. The initial trials were carried out in small interval to get the control over etching process and after couple of trials protocols were established successfully.

## 6.3 Second Photolithography with negative photoresist

### 6.3.1 Importance of negative photoresist thickness and uniformness

After removing the photoresist, the next step was the deposition of negative photoresist Az- 125 nxT-10A which is a viscous compound and having 1-Methoxy-2- propanol acetate as main component as discussed in material data sheet AZ 125nXT-10A Photoresist.

This photo resist was selected due to its high mechanical strength and stability to endure the harsh environment. it was essential so that it could tolerate the high intensity through hole etching process and preserve the substrate material and structure where it would behave as a protective mask against aggravated etchants. That is why the thickness of

the photoresist was vital and at the same time too thick film could also cause difficulties in exposure and developing steps. So, the thickness of the resist was regulated as the trials proceeded. Similarly, the uniformity of the film was also an important factor. Due to highly viscous nature it was evident that it would spread unevenly on substrate surface. Uneven uniformity means there would be different thickness of photoresist across the wafer surface and it would cause the different regions of the wafer would absorb more light than others and consequently its response to exposure and development would be non-uniform . The regions that absorbed less light was not developed fully or they yield more thicker and wider geometries as compared to other parts which absorbed more light.

The other potential problem which was encountered during the deposition was the formation of the bubbles. These bubbles appeared due to voids between the layers of photoresist. To encounter bubble formation the resist was dispended in the centre of the wafers and let it spread evenly. In addition, some bubble disappeared also during soft baking.

### 6.3.2 Importance of Soft bake with negative resist

After depositing the resist, the wafers were baked for 60 minutes at a temperature of 130°C. It was important to get the uniform thickness of the resist and to evaporate the solvent present in the resist. In addition, it also ensured that the resist did not contain any cracks and had the minimum mechanical stresses. The timing and the temperature were quite sensitive for the process as little bit over heating burnt the resist and got stiff on the wafer surface which would have adverse in following exposure step as the pattern couldn't transfer properly . it also took longer time to get it off from wafer. All such cases were wasted

### 6.3.3 Alignment and exposing with negative resist

Alignment of the second layer with first layer posed the serious challenges. It was tough to align the mask pattern very precisely due to thick layer of photoresist and very minute size of the geometry. At the same time, it was time consuming to find first etched pattern and alignment marks because the thick negative photoresist confined the focusing distance and minor misalignment could damage the entire chip. There were three

alignment marks on the mask as can be seen in the figure 3-13 and 3-14 and all of those require to be fit ad coincided with each other to get the perfect alignment.
The dense nature of the negative resist required high energy dose and was calculated as

$$Energy\ dose = exposing\ time\ \times power\ density\ of\ UV\ \ light$$

$$12.5\frac{mj}{cm2} \times 600\ sec = 7500\ mj.\frac{sec}{cm2}$$

During the trial, the exposure time was tried with different intervals. Due to thick film it was obvious that exposure time would be longer than 300 seconds. it was tested for time span of 350, 400, 450 , 500 , 550 , and 600 seconds and the accuracy was found at 600 seconds.

### 6.3.4 Challenges during second developing

For the negative resist, the portion of resist exposed to UV light become more hard or insoluble and while developing the insoluble resist remains on the substrate surface and non-exposed region dissolves. The clean removal of the resist was important so that sidewall of the structure would remain straight which was necessary to achieve better delicate through hole etching. As expected it would take longer time to develop so various time interval were tested between 200sec to 400 seconds and the appropriate time was different for different wafers to be on a safe side the developing process halted first at 200 seconds thereafter for every 30 seconds accordingly and inspected into the microscope. On average the developing time was set about 300 seconds. The most probable reason for the varying developing time could be variations in film thickness.

## 6.4 Through Hole etching and challenges

Through hole etching was a complex and intense procedure. It required high power and generated a lot of heating. During initial trials it was discovered that the thick and viscous negative photoresist sticks with the etching clamp and no wafer come out when the procedure is completed , then we had to open the etching chamber to get it out manually which was time consuming . To overcome this, it was suggested to use a polyamide tape to cover the annular edges to avoid such attachments. Secondly, As the high-power etching progress the through holes appears and become a window which means the

plasma ions will undercut the photoresist mask. Such undercut destroyed the pattern very fast.

The initial tests performed in a one big cycle and the duration of the procedure was about an hour. The continuous operation of etching for an one hour also broke many wafers from the edges and some wafers even broke completely in pieces and this scenario was the worst as manually cleaning and cooling wasted time and most importantly a well patterned wafer was lost . The long operation also caused over-etching frequently which means the important pillars of the structure were damaged due to longer duration of operation. To prevent this situation initially a $SiO_2$ is attached but this strategy also could not work to avoid wafer breakage.

To overcome over-etching and breakage of the wafers it was decided to do the whole process in two or three short intervals and after every interval it was tried to measure the depth of the cavity produced in the wafer and according to the measurement the parameters were regulated in the following procedure . The short interval also allowed to stop the operation in between and cool down the wafer as well as the chamber. The recipe for the short intervals were adjusted so that in first cycle was supposed to do wide intense etching on the structure and later intervals were incorporated such that they would etched relatively slowly and in a shallow way .Even though this strategy also damaged some wafers but its success rate was much better than previous results. At the end of the dry etching only five wafers out fifteen produced the desired results.

### 6.4.1 Anode Bonding

Anodic bonding is a very mature technique which is employed for wafer packaging and wafer stacking. Anodic bonding is utilized mainly to bond silicon wafer with glass wafer having high composition of alkali oxides. On the trial basis Pyrex glass and silicon wafers were brought together in contact under an external voltage and temperature because it has similar kind of characteristics as actually fabricated borofloat 33 glass. Anode bonding of three wafer can be done in either two or three steps but the later has the advantage for a smaller number of process steps and increased throughput.

### 6.4.2 Three wafers bonding

The purpose of the three-wafer stock was to prevent the thermal shock for sensitive etched microchannels. The other main reason of introducing three wafers stack was to avoid high bow appearance which happens in two wafer bonding (Si-glass ) because of thermal mismatch of both the materials and by making the exterior of the stack with same material prevent this problem . In addition, the three wafer were critical for the lure and tube connection network incorporated in the device. Lastly the upper glass also provides a transparent layer to monitor the actual process and assist to regulate the flow [27].

### 6.4.3 Challenges during Anode Bonding

There are some parameters which influence the whole bonding process; among those temperature, force and volage play the significant role. During the very initial trials the parameters were held constant at temperature 500°C force 2500N and voltage at 500V. Such tests provided the approach to proceed the process. The first issue which was realised that bonding efficiency would be enhanced if step voltage would be applied rather than constant high voltage. Step voltage allowed us the voltage must be increased in short intervals in order to minimize the high current flow which produce electrical discharges and allowed movement of metal ions towards cathode. For this purpose, the voltage of 700 V was applied in three equal steps of 12 minutes. It showed some improvement in test 1 though it was not satisfactory. In later tests the same approach of step voltage was employed with different values and it was observed that increasing voltage had a positive impact on the bonding process. Similarly, the pressure which was exerted on the stack was measured as force increased in different tests and it was observed stronger force was also favourable for better contact and bonding.

The other biggest challenge and the most important factor in bonding process was the cleaning of the wafers. Contamination present on the surface of wafers were the major cause for the unsuccessful and improper results during the process. Therefore, the cleaning process was emphasised to prevent accumulation of any foreign particles on the surfaces of substrates and to remove the native oxide present on the wafer surface. For the trial_purposes we used the plain wafers which mean they were already reasonably cleaned but still they offered many difficulties during the procedure. The actual wafers

with structure were subjected to have many kinds of contaminations as they had undergone many harsh chemical environments. It was suggested to do the contamination test for the wafers which have structure to modify the cleaning strategies, but that facility was not available locally. Along this the uniformity of the structure wafer is an issue and it can offer potential difficulties for silicon and glass interface .It will be a bigger challenge for patterned wafers as they have holes and elevated features as well as aligning of structed wafer would be an potential issue .

Along the trapped particles (which are the one of the root cause of the voids in bonding process ) the electric field nonuniformity was also another reason for the unbonded area between the two wafer .Since the metal ion diffusion takes place at the interface of both surfaces which depends on the generation of electric field. For nonuniform electric field these metal ions begin to accumulate on the wafer surface and prevent bonding on these regions.

The size of the wafer might be another factor which reduced the bonding efficiency. For that particular case both the wafers were of same size (100mm)which mean there were slight shift of the wafer edges and it would be difficult to create the two contacts .In that case it was hard to avoid the electrical discharges or shortcuts which refrain from acceptable bonding process . Therefore, the glass with bigger in size than silicon wafer and have metallization would be preferable.

A study was conducted in which the trials for triple stack (glass-Si-glass) anode bonding was carried out [27]. Same cleaning techniques were employed as stated above and the dimension of the wafers were also the same with plain surfaces. The three wafers were loaded simultaneously and the temperature of 420 and step voltage of 600 V was applied. According to authors there were no big voids, and no unbounded area was found in particular as can be seen in figure 6-1.

In comparison to our work there are some concerning areas which might be the factor for the difference in results. Like the author has neither mentioned the type of the glass used in experiment nor the cleaning time. In addition, equipment model and the total process time was also different as compare to our work.

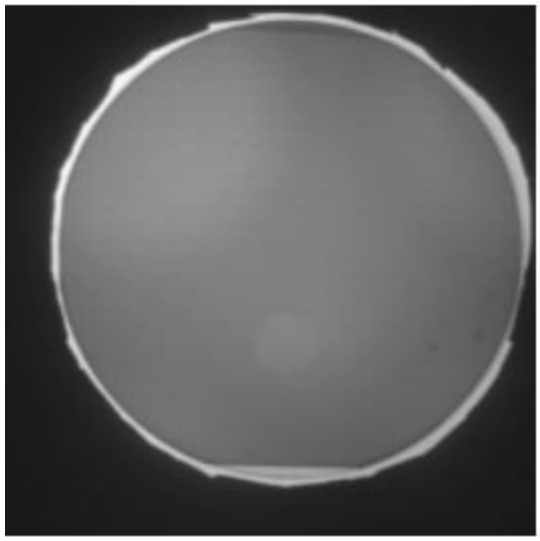

*Figure 6-1 the IR transmission image shows Glass-Si-Glass bonding (courtesy of the work done by V.Dragoi , T.Glinsner , P.Hangweier and P.Lindner) [27]*

Unfortunately, the last step in fabrication of the device could not be completed as planned. Many meetings have already been conducted in this regard both internally and externally and there are many options which are under considerations. For example, it can be done entirely by third party but at the same time it is also necessary to get the expertise internally for future assignments. Secondly some experts can be hired who have been involved in these practices and learn from their experience and protocols can be established. Lastly, the whole process can be carried out at the USN vicinity by local researchers and lab engineers which might take 1-2years further.

# 7 Future Work and Conclusion

## 7.1 Assembly and Validation

The first and prime focus will be the assembly and packaging of the all the completed wafers through anode bonding which is offering a serious and major challenges right now. Once it has been done then the testing of the device will be carried out and its performance will be evaluated. Generally testing procedure is being executed in different phases. For this purpose, it will be accomplished in two phases in order to evaluate the separation efficiency of the device in which various technical parameters under different operating environmental conditions will be strictly assessed for surveillance of any potential operational safety risks and other technical problems .The first testing will be done to determine the operability and functionality of the device(s) and its components. In this phase the goal will be to separate and concentrate micro algae directly from water. If the device(s) passes the first test, then it will be subjected to a second test then we will try to separate and concentrate all microorganisms in crude oil.

## 7.2 Diagnostic Kit

At present the world is facing new challenges related to COVID-19 (60–140nm) pandemic which has not only threatened the life of every single individuals but also damaged the world economies. The primary issues which authorities are facing is to stop the widespread of virus by keeping the distance among people and the early diagnostic of the virus. PASECOR has promised to revolutionize the medical sectors too and in this regard study is under way how it can play its rule in recent challenge .In the future the PASECOR technology will be able to produce cheap and easy operated home diagnostic kit by separating the virus from saliva or from air [28].

## 7.3 Upgrading

To separate smaller organism which are below 5µm the device the constitution of the device will have to changed. For example, the distance between the pillars can be reduced up to 1µm or even further by employing advance nanofabrication techniques.

This will ultimately broader the applications of the PASECOR and open new doors for research.

## 7.4 Commercializing the technology

After successfully completing the TRL level 4,5,and 6 the manufacturing companies within the PASECOR Centre will further develop and produce the pilot plants into TRL level 7, 8 and 9 in which process and production industry will be motivated to fund and support the development of standardized full scale plants .

## 7.5 Conclusion

The design and fabrication of the microfluidic chip has been successfully accomplished. A fully integrated PASECOR chip with micropillar arrays was realized and attained results with discussion have been provided. During fabrication, many challenges have been faced in order to design implementation. Those challenges are provided in details and target to prepare the number of chips was achieved successfully. The outcome could be more satisfactory if it could be managed to assemble the chips through anode bonding as discussed in 3$^{rd}$ objective. The remaining work on anode bonding will be pursued in future.

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# List of Figure and Tabels

Figure 5-1 Figure 5-1 displays the pillar structure arranged on the elliptical profile. The image was taken by Leica Optical microscope with bright field mode and 5X, 10 X magnification

Figure 5-2 Figure 5-2 was taken with Lecia Optical microscope in bright field mode and 20X magnification which shows the pattern of pillars arrangements

Figure 5-3 Figure 5-3 was taken by Profilometer DEKTAK 150 to measure the thickness of photo resist after developing photo resist, the x-axis is the length of scanned area in[µm], y-axis is the information about height or thickness of photoresist in [µm]

Figure 5-4 Figure 5-4 Thickness measurement has been performed by Profilometer DEKTAK 150 to measure the thickness of photo resist after developing positive photoresist, the x-axis is the length of scanned area in[µm], y-axis shows information about height of photoresist film in [µm]

Figure 5-6 Figure 5-6 Missing structures during first lithography. these figures were taken with Lecia Optical microscope in bright field mode and in different magnifications.

Figure 5-7 Figure 5-7 Missing structures during 1st lithography. these figures were taken with Lecia Optical microscope in bright field mode and in different magnifications

Figure 5-8 Figure 5-8 Line width of the structure during first photolithography. The image was taken with Leica mobile in different magnification

Figure 5-9 Figure 5-9 Measurement performed by *Profilometer DEKTAK 150* to measure the height of fabricated pillar with respect to silicon substrate after small interval of dry etching process ,the x-axis is the length of scanned area in[µm], y-axis shows information about height of pillars [µm]

Figure 5-10 Figure 5-10 Measurement has been performed by *Profilometer DEKTAK 150* to measure the height of fabricated pillar with respect to silicon substrate after dry etching process, the x-axis is the length of scanned area in[µm], y-axis shows information about height of pillars [µm]

Figure 5-11 Figure 5-11 Measurement has been performed by *Profilometer DEKTAK 150* to measure the height of fabricated pillars with respect to silicon

## Tables

Table 5-5 Table 5-4 shows the parameters used to measure the height of the pillors

Table 5-6 Presents the parameter used to measure the height of negative resist after developing