

Introduction

- Momentum, mass and energy transport in sub-millimeter sized system or channel under a driving force.
- The subject received significant attention over last decade due to miniaturization of sensors, biomedical implants, making of novel materials and structured catalyst
- Lab on chip: Entire bio/chemical laboratory on the surface of silicon or polymer chips
- Leverages the techniques that are developed over years for silicon based microelectronics industry.
- Polymer based lab on chip is cheaper and easy to produce in mass scale.

Scaling laws

- Surface forces (surface tension gradients and shear forces) is proportional to $(\text{length})^2$.
- Volume force (e.g., gravity and inertia) is proportional to $(\text{length})^3$.
- Their ratio tends to infinity as the length scale is reduced.
- Volume forces become unimportant, and surface forces dominate in microfluidic scale

Continuum hypothesis

- Fluid is not perfectly continuous in structure, instead composed of several molecules.
- Physical quantities e.g., mass, momentum and energy associated with small volume of fluid containing large number of molecules are taken as sum of corresponding quantities for the molecules in the volume.
- At atomic scale (e.g., probe volume in AFM), large fluctuations due to molecular structure of fluid become significant.
- At larger probe volume with sufficiently large number of molecules, well-defined average values with small statistical fluctuations are obtained.
- A liquid cube of 10 nm side contains 4×10^4 molecules and exhibit 0.5% fluctuation in numbers. For gas, the size is 10 times.
- For probe volume larger than 10 micrometer, variations in physical properties due to external forces appear.

Eulerian formulation

- Definition of field variable
- Measurement of velocity using microparticle image velocimetry
- Microparticles with diameters of the order of $1\text{ }\mu\text{m}$ are suspended in the flow.
- Positions are recorded as grey-scale values in CCD camera through an optical microscope, using either transmitted or reflected light.
- Two pictures are recorded by sending two light pulses in quick succession of millisecond apart.
- Use of cross-correlation function to determine average velocity at point r .

Lab on a chip application

- Pharma (R&D in drug development, quality control, screening)
- Environment (water monitoring, pollution)
- Medical (Allergen detection, toxicology, implantable drug delivery system, flow meters in biomedical implants, tailored medication)
- Life Science (Diagnosis of disease, gene identification)
- Industrial Pollution Control (Bioreactors, food composition to check pesticides and antibiotic residue)
- Forensic medicine

Other applications

- Microreactor
- Contacting in extraction
- Heat exchanger in electronic circuits
- Microdroplet as templates

Advantages of lab on chip

- Shorter time
- Upfront capital investment – reduced
- Close to real time measurements (Blood, food, effluent samples)
- Small requirement of samples – smaller chemical footprint
- Digital output and interfacing with software
- In drug discovery, small volume of expensive reagents, parallel operation, high throughput screening, reduced human error

Advantagescontd.

- High surface to volume ratio → Increased catalytic activity and better heat dissipation for highly exothermic reaction → reduced coking, thermal run-away, sintering.
- Robustness of the reactor
- Hazardous reaction at the point of use
- Difficult reaction that requires precise control, and otherwise attainable in a randomly packed bed (e.g., propellant from CO₂ in space-ship)
- Others

Components of lab on chip

- Pumping
 - Centrifugal force
 - Surface force
 - Electrokinetic force
 - mechanical
- Valve
 - Hydrogel
 - Hydrophobic layer
 - Mechanical
- Separation
 - Field flow fractionation (electrical, thermal, flow)
 - Electrophoresis, Dielectrophoresis, DEP + FFF
 - Diffusion based separation (H-Filter)

Components of microfluidic device ..contd.

- Mixing
 - Passive using grooves, laminations
 - Active
- Diffusion between layers
 - T-Sensor
- Heating
 - Cyclic heating for PCR reaction – DNA hybridization
- Detection
 - Optical interrogation
 - Amperometric sensing

Flow is laminar and the interaction between layers are utilized in most of these components

Pumping

- Use of moving parts as in a conventional pump with the help of micromachining.
- Centrifugal force to drive fluid through channels in radial direction (lab on CD).
- Use of coating with favorable contact angle, and pillars in the channel to enhance a “capillary rise” type flow.
- Electro-osmosis: Polar liquid in contact with solid wall induces surface charges, which in turn influences migration of charges within the liquid near the wall. Voltage gradient along the length of the channel pulls the charges, and the bulk liquid along with it.
- Electro-wetting: The change in contact angle of a droplet on a surface when an electric field is present at an interface. A droplet is held between two sets of planar electrodes (upper one consists of single continuous ground electrode, and bottom one with an array of independently addressable control electrodes). By spreading the droplet using the electric field such that droplet touches adjacent electrode in the array, and then switching on the adjacent electrode – movement of droplet is accomplished.

T-sensor

- Sample Stream:
 - Sample antigen to be measured (SA)
 - Fluorescently labeled antigen (LA) kept to a concentration 2-3 orders of magnitude less than anticipated SA concentration
- Other Stream:
 - Known concentration of antibody (AB) to the target analyte

AB molecules are larger and slow to diffuse

1. AB binds with all LA if SA is not target analyte → Bound LA cannot diffuse into AB stream → color stays near interface
2. AB binds with SA and some LA if SA is target analyte → Lot of free LA and free SA to diffuse into AB stream → spread of color into AB stream.
3. More than one T-sensors in the chip with different AB to target analyte.
4. Spread of color can be determined digitally.

H-filter

- Two parallel laminar streams will flow. One is the sample stream (e.g., blood containing aggregates of different sizes). The other is acceptor reagent (e.g., saline water).
- Smaller molecules diffuse faster.
- Smaller components of the sample stream will diffuse into the acceptor stream.
- At the end of the channel, two parallel flows are split up into two reservoirs.

Detection

- Laser induced fluorescence system
 - Fluorophores are conjugated with migrating analytes
 - Laser beam excites the fluorophores
 - Resulting fluorescence signal is filtered to block background illumination from the excitation source.
 - Fluorescence signal is recorded using CCD camera, PMT, APD
- Electrochemical
 - Monitor variation of electrochemical potential as analytes migrate past a working electrode, positioned within the separation channel.
 - The conductivity is related to the concentration of species.

Electrophoresis

- Migration behavior of charged species under the influence of an electric field.
- Analytes are suspended in an ionic buffer environment at a specific pH
- Each species migrates with a different mobility, allowing them to be resolved as distinct zones, and separated on the basis of size and charge.
- Biological macromolecules (e.g., proteins) are analytes
- Drag and electrophoretic mobility are the counteracting forces. Gravity is neglected.
- In some cases, polymer gel acts as sieving matrix material in the separation channel. The gel matrix reintroduces a size-dependence to the electrophoretic migration.
- In gel electrophoresis, analytes travel through the porous gel network with smaller fragments experiencing less resistance and eluting faster

Dielectrophoresis

- Particles are separated based on dielectric properties.
- A non-uniform electric field is generated by the use of planar and point electrodes.
- More polarizable particle will be attracted to strong field region.
- DEP can be used as a trap or in FFF mode. In the second case, gravity acts against DEP forces in settling the particles at a particular height in the channel.
- Live cells can be separated from dead cells by this method.

Electrical Field Flow Fractionation

- Top and bottom walls of the channel comprise of electrodes. Voltage is applied lateral to the direction of flow.
- Particles with high electrophoretic mobility will pack more closely to the wall.
- Flow in the channel is laminar, and easily characterized (e.g., parabolic).
- Each layer moves at different velocity, and particles carried by the layer elutes at different times from the outlet.
- Slugs of particles, separated based on their size/charge is detected at the outlet, and identified based on prior calibration.

Other modes of FFF

- Flow FFF

- Use of transverse flow with membrane attached to wall
- Concentration polarization directs the particles to settle in a layer depending on the diffusivity (size).
- Laminar flow carries the particles at each layer to downstream with different residence time

- Thermal FFF

- Diffusivity is a function of temperature and particle size
- One of the channel walls is hot, and the other wall is cold – thus creating a thermal gradient across the channel.
- Higher molecular weight particles compact more tightly against the cold wall than do lower molecular weight particles.
- Because of laminar velocity profile of the carrier, lower molecular weight samples will have a higher average velocity, and elute before higher molecular weight samples – resulting in a fractogram.

Valve made of hydrogel

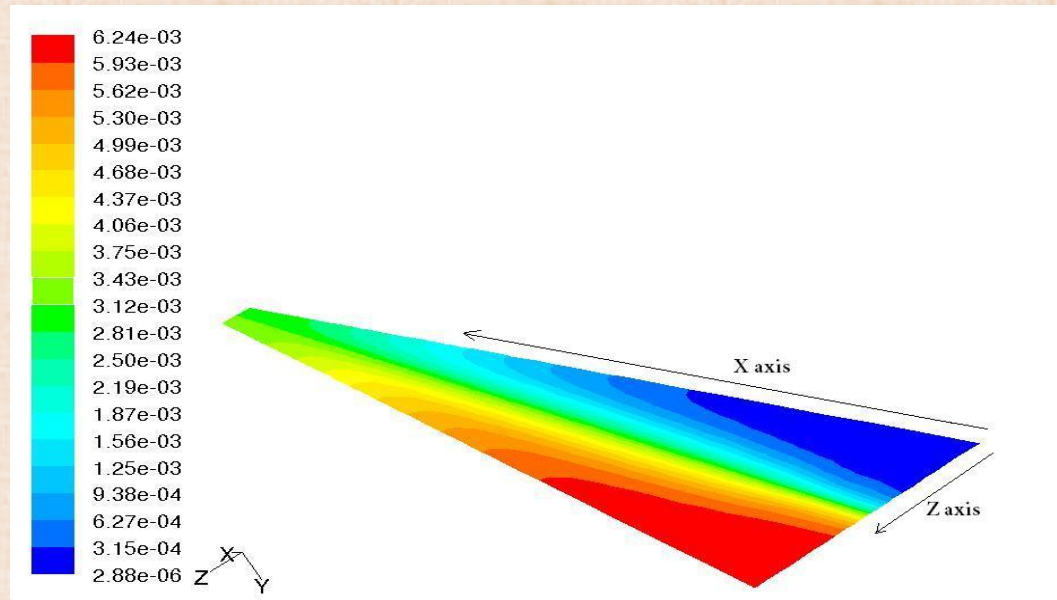
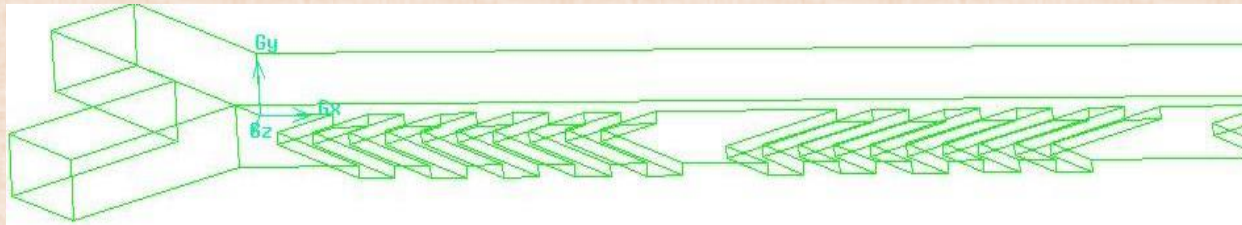
- Posts on the channel with hydrogel jacket around it.
- Hydrogel in its expanded state, block the channel.
- Contracted hydrogel allows the flow of fluid down the channel.
- Expansion / contraction by external trigger (pH or thermal)
- Response time is critical. Fractional change in diameter should reach 1.0 within seconds.
- Time response can be tailored by selecting the right number of posts and the thickness of the hydrogel layer.

Valve made of hydrophobic layer

- A liquid being unable to expand freely, forms an interface with second liquid or gas.
- If interface is curved, there is pressure difference across the interface. The pressure is higher on the concave side. The pressure increase is balanced by surface tension forces.
- For flow to take place, the applied pressure gradient has to be greater than the capillary pressure.
- An abrupt change in the width of the channel causes pressure drop at the point of restriction.
- By adjusting the width and height of the channel at the constriction, and with wall coating that provides adverse contact angle, it is ensured that flow can take place only when pressure at upstream side exceeds pre-specified value.
- Similar concept can be used for flow splitting.

Passive mixing

- Diffusion between two parallel streams in laminar flow – enhanced interface between the streams.
- Two streams are allowed to intermingle, brought close to each other, and then depart from each other due to baffles in the channel.
- Cross-flow between two streams is induced by inclined grooves.



Microfabrication

Fabrication accuracy

City (10 km)---House(10 m)----Optical fiber (1 mm)----Bacteria($1\mu\text{m}$)----Virus($0.01\mu\text{m}$)--- 1\AA

Traditional mechanical machining

- Object of size 1mm to 50 cm
- Precision of the order of $10\mu\text{m}$

Special mechanical machining

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Special mechanical machining

- Displacement precision $\sim 1\text{ nm}$
- Angular precision $\sim (10^{-5})^\circ$
- Applicable for metal alone. Not for glass, teflon or plastic
- Price tag

Etching / lithography / deposition

- Range $0.2\mu\text{m}$ to $500\mu\text{m}$
- Use glass or silicon

Microfabricationcontd.

Plastic MEMS

- Range 0.5 μm to 500 μm .
- Use elastomers such as PDMS (polydimethyl siloxane) or PMMA (polymethyl methacrylate).
- Channels can be made by either direct method (laser ablation) or replication method (use of mold).
- By weight, plastics are 100 times less expensive than silicon, and can be made disposable.
- Rapid prototyping.
- Surface effects, transparency, diversity of materials can be introduced easily.

Clean Room

- Temperature regulated at 20°C.
- Humidity regulated and traversed by flux of filtered air.
- Number of particles of size $< 4 \mu\text{m}$ per cubic inch defines a clean room.
- For fabrication of microprocessor, a number ≤ 10 is suggested.
- For MEMS microfabrication, a number $\leq 10,000$ is suggested.
- Wearing hair-cover, shoe-cover, gloves required in the second case also.

Photolithography mask

- Protects certain part of a resist from illumination (spectral band 300 nm to 450 nm).
- Plates of quartz on which deposits of chrome forms a pattern.
- Deposition is made using electron beams.
- A less-precise one can be just a print-out on plastic transparency sheet.

Photosensitive resist

- Mask is placed on top of a substrate, covered by photosensitive polymer.
- Substrate is put under uniform beam.
- The regions appear on the polymer as lit or dark as per the pattern that had been designed on the mask (pattern transfer step).
- Light sets changes in photosensitive polymer and make the polymer soluble to certain solvent. Upon dipping in the solvent, the polymer in the lit portion is dissolved.
- The exposed portions of the substrate undergo chemical attack from the etching fluid.
- Depending on the time over which the substrate is kept in etching fluid, the exposed portions are etched to a depth.

Spin Coating

- Photosensitive polymer is deposited in a thin layer on a solid substrate of silicon or glass.
- Three stages of the coating process are Distribution, Spreading, Evaporation, Solidification.
- Use of vacuum to hold the wafer.
- Equilibrium thickness: The thickness achieved after spinning for few minutes. With evaporation of solvent, viscosity increases. With decrease in radial velocity, viscosity increases for shear-thinning fluid. The fluid stops moving beyond a distance.
- Heating of resist at 70°C for few minutes to evaporate 15% of solvent remaining to avoid formation of cracks.

Air bubbles in dispensed resin

Dispense tip is cut unevenly or has defects

Dispense rate is too high

Spin bowl exhaust rate is too high

Resist on wafer for too long prior to spin

Spin speed / acceleration is too high

Fluid is striking substrate off centre

Spin time too short

Insufficient dispense volume

Air bubbles / particles present in resin

Particles on substrate prior to dispense

Exposure

- Substrate, coated with photosensitive polymer is placed in an aligner.
- Luminous flux crosses the mask and hits the coating on the substrate.
- Light of wavelength 300 – 450 nm is used.
- Luminous flux initiates reactions in the polymer.
- Positive Resist (PMMA): After exposure, the polymer becomes soluble in a solvent as the light weakens the internal bonds → Exposed part is eliminated.
- Negative resist (KTFR from Kodak, SU8 from IBM): After exposure, the polymer becomes insoluble in a solvent, as light induces covalent bonds between chains → The unexposed part is eliminated.

Other issues

Half-light effect during exposure

- Opaque object produces shadow as well as penumbra.
- Diffraction.
- These effects should be as small as possible.

Transparency of the resist

- Light has to penetrate and modify the resist over the entire thickness of resist layer

Choice between positive and negative resist

- Negative resists adhere better to the substrate.
- Negative resists are more chemically resistant.
- Positive resists show better contrast in photosolubility.

Typical Protocol

- Immerse the wafer in acetone in an ultrasonic bath to dissolve organic residue and then drying.
- Repeat the previous step with alcohol.
- Dehydrate on a hot plate at 120°C for 5 minutes
- The first three steps, above ensure removal of contaminant from the wafer.
- The wafer is coated with photoresist in spin-coater.
- System is heated to 65°C for one minute, and 95°C for three minutes to ensure hardening of photoresist.
- Exposure.
- The wafer is post-baked to 65°C for one minute, and 95°C for two minutes to ensure progressive rearrangement of material during thermal deformation.
- The system dipped in developing solution for 3 minutes.
- The system is rinsed with alcohol, and dried with nitrogen.
- The system is heated to 200°C for two hours.

Silicon

- Delivered in the form of wafer that constitute a monocrystal.
- Produced by well-controlled crystalline growth process in clean-room (Class 1-10), by slowly pulling a crystal from an ultrapure bath of silicon.
- Slices are cut from a cylinder, followed by atomic polishing phase.
- Typical thickness of wafer $\approx 500 \mu\text{m}$.
- The other dimensions ≈ 4 inches.
- Two inter penetrating face-centred cubic lattice network.
- Side of one cubic face is 5.43 \AA .
- Highest density planes $\langle 111 \rangle$ form an angle of 54.74° to $\langle 100 \rangle$ plane.

Wet etching

- Subjecting parts of an object to chemical reactions and subsequent dissolution.
- The other parts are protected by a mask.
- Technique is used in fifteenth century to decorate armours. Wax is used as a mask. Wax on parts, identified for etching is cut out. Dipping into reactive bath for a period of time ensures chemical etching / hollowed out pattern in places, not covered by wax. Wax is removed by heating later.
- Rate of etching follows Arrhenius expression, and is strong function of temperature, unlike solubilisation of resist.

Anisotropic etching

- $\langle 1\ 1\ 1 \rangle$ planes of silicon are highly packed with atoms.
- Velocity of etching is slow along planes $\langle 1\ 1\ 1 \rangle$ and fast along $\langle 1\ 0\ 0 \rangle$ or $\langle 1\ 1\ 0 \rangle$.
- Typical etching speed with KOH along $\langle 1\ 1\ 1 \rangle$ plane is $13\ \mu\text{m}/\text{hour}$.
- The etching speed is 60 times faster in other orientations.

Anisotropic etching ...contd.

- Etching happens preferentially along a crystallographic plane.
- The crystal will make the cut-out forms appear spontaneously along the planes where the etching is slowest.
- This form of etching makes possible cavities with facets, which can be useful.
- This type of etching is not possible in amorphous solid, e.g., glass.

Isotropic etching

- Carried out equally in three spatial direction.
- Example: Etching of glass by HF.

Dry etching of silicon

- Attack of a substrate by an ionic species, contained in gaseous or plasma phase.
- Isotropy or anisotropy controlled by the etching process, and not by crystalline structure.
- Types of dry etching:
 - Physical etching (sputtering, ion-beam etching, ion milling)
 - Chemical etching
 - Physico-chemical etching (RIE)
 - Physico-chemical etching with inhibitors (DRIE)

Physical etching

- Ions are accelerated in an electric field (10 eV to 5000 eV generated between two electrodes).
- Ions bombard the surface of a target.
- Low pressure (\sim mTorr) helps in making the ion ballistic.
- Use of CF_4 gas is common in ionization.
- The method is inherently anisotropic because of the pathway taken by the reflected ions.
- Selectivity (about the portions of the substrate to be etched) is poor.
- Etching rate $0.6 \mu\text{m}/\text{hour}$ to $18 \mu\text{m}/\text{hour}$ for most materials.

Chemical etching

- Chemical species (neutral) migrate towards the target by diffusion.
- Chemical reaction occurs on the surface of the target.
$$2\text{XeF}_2 \text{ (sublimated)} + \text{Si} \rightarrow 2 \text{Xe} + \text{SiF}_4 \text{ (volatile)}$$
- Both the products desorb and diffuse back.
- Chemical species not accelerated by application of voltage.

Chemical etching ... contd.

- Volatile products are not reflected.
- Movement is diffusive, and not ballistic.
- Mostly isotropic etching.
- Etching rate $\approx \mu\text{m}/\text{min}$.
- Etched surface is rough.
- Pressure is maintained at 0.1 Torr to 1 Torr.

Physicochemical dry etching (Reactive ion etching: RIE)

- Combined effect of physical and chemical etching.
- Commonly used in microfabrication.
- Target is placed on the cathode.
- Cold plasma breaks down CF_4 to $\text{CF}_3^+ + \text{F}^-$ and makes the ion ballistic at low pressure.
- Anisotropic; Etch rate 10 times that of physical etching.
- Good in etching SiO_2 (wet etching of SiO_2 takes time) from the surface of Si wafer.

Physico-chemical etching with inhibitor

(Deep reactive ion etching: DRIE)

- Physicochemical etching with non-volatile reaction products.
- The gas is CCl_4 .
- Non-volatile component (inhibitor) is deposited on the neighbouring vertical surface.
- A polymerized protective film is formed on the vertical surfaces.
- The film protects the vertical surface from the reflected products, arising from ballistic ions → Very deep structure $\sim 500\text{ }\mu\text{m}$ with aspect ratio of 30:1 can be obtained.
- Making a through hole of high aspect ratio is difficult from wet etching due to anisotropy.

Deposition on silicon and glass

- Deposition is required to form electrode, catalyst, thermal or electrical insulation, adsorbent, optical elements on the wall of the channel.

- Physical vapor deposition

Certain species from the holding gas is adsorbed on the surface of the target. Two types are thermal evaporation and sputtering.

- Chemical vapor deposition

Certain species from the holding gas reacts with the target forming compounds that are chemically bonded to the target.

Thermal evaporation

- Material to be deposited faces the target in a container.
- High temperature ensures sublimation of materials.
- Low pressure (10^{-8} Torr) ensures avoidance of unwanted parasite deposition of molecules present in the chamber.
- Deposit rate $\sim \text{\AA}/\text{s}$
- Simple to implement. Good for metal deposit.

Sputtering

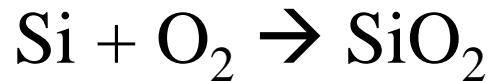
- Target is on anode, and the material to be deposited is on cathode.
- Use of cold plasma gives the option of depositing variety of materials.
- Ionic kinetic energy of 0.3 keV to 2 keV ensures penetration by one or two molecular layers of the substrate → good adhesion.

Chemical vapor deposition

- Homogeneous reaction takes place in the gas phase, and the product gets adsorbed on the target surface.



- Heterogeneous reaction at the surface of the target.



2 nm layer of SiO_2 forms when silicon wafer is exposed to ambient air.

The thickness of SiO_2 will be $1 \mu\text{m}$ when exposed to air at 650°C .

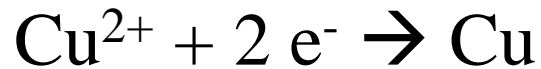
- Gas phase diffusion ensures low defect density in crystal.

Chemical vapor depositioncontd.

- Three types of CVD: LPCVD (low pressure ~ 1 Torr), APCVD(atmospheric pressure), PECVD (plasma enhanced, low pressure).
- Plasma enhances chemical reactions at the target surface using ionic bombardment. Also, energy of incident particles helps penetrating into the target surface, resulting in constant film thickness and conformal deposition.
- Lower pressure ensures that the mean free path of molecules is high for easy diffusion of species.
- Deposition rate $\sim \mu\text{m} / \text{hour}$.
- Versatility in material deposited (includes deposition of insulators)

Electrolytic deposition

- Metal deposition on the substrate held as anode.
- Solution is metallic salt (e.g., CuSO_4).
- Metal ion (Cu^{2+}) migrates towards anode.



- Metal ion captures electron at the electrode and form molecule that adsorbs onto the electrode.

Sealing in silicon and glass

- Grooves, curved onto the silicon wafer must be covered with another glass wafer in water-tight manner to complete the making of device.
- Anodic bonding refers to glass-silicon sealing by the use of intense electric field (1 kV) and elevated temperature (400°C).
- The electric field induces migration of Na^+ ions in the glass to the interface, and interpenetration of atoms at the glass-silicon interface.
- Thermal expansion coefficient of glass lid should be same as silicon to avoid crack. Pyrex Corning 7740 may be a suggested one.

Sealing in silicon and glass

- Cleanliness of surface is important.
- Anodic bonding between silicon to silicon is possible through intermediate glass layer. Thin glass layer (0.5 μm to 4 μm) is deposited on silicon by physical vapor deposition or spin-on technique.
- Seal from anodic bonding is water-tight up to 100 atmosphere.
- Fusion bonding refers to bonding Si-glass wafers without application of any voltage, instead using higher temperature (600°C to 1100°C).

Fabrication of plastic microfluidic devices

Moulding, Casting, and Microinjection

- Mould is the negative master, made of silicon, electrodeposited metal or reticulated polymer.
- Common method of making a mould is photolithography. For processing at high temperature and pressure, electrodeposited or traditional micro-machined metallic moulds are used.

Moulding

- Catalyst + polymer is poured on a mould and heated.
- Later, the structure is peeled off the mould and contains the pattern of the mould in negative form.
- Common polymer is PDMS.
- Temperature of about 70°C is required.

Casting / hot embossing

- Mould / Stamp is pressed into a heated deformable material.
- After cooling and separation, the structure represents negative of mould.
- Common polymeric material is PMMA. Other plastics are polyethylene, PVC, PEEK.
- Temperature $\sim 170^{\circ}\text{C}$
- Pressure $\sim 10 - 100$ bar.

Microinjection

- Meant for serial production on industrial scale.
- The liquid plastic material is injected into a mould under a vacuum at a temperature $>$ Glass Transition Temperature.
- Next, the system is cooled down below the glass transition temperature.
- Mould is taken off, and a structure corresponding to the negative of the mould is obtained.

Advantages of plastic microfluidic devices

- Precision is surprisingly high (sub-micrometric precision).
- PDMS is transparent.
- Elastomeric quantity helps water-tightness by holding on tightly to the lid / interconnect.
- Weak surface energy helps in peeling from the mould.
- PDMS is permeable to gas. Trapped air can escape during liquid fill-up operation.
- Untreated PDMS is hydrophobic. It becomes hydrophilic after oxidation of methyl group at the surface by oxygen plasma.

Disadvantages of plastic microfluidic device

- Aging
- Deformability for high aspect ratio.
- PDMS cannot tolerate high temperature → evaporative deposition of metallic electrodes is not possible. However, hybrid structure made of PDMS layer placed on silicon wafer with evaporated electrode is possible.

Direct micromachining of plastics

- μ -stereolithography: photosensitive resists are polymerized layer after layer by applying a spot of UV laser.
- Laser ablation: Plastic is removed locally by sublimation using intense laser beam.

Bonding of two wafers to put a lid on carved microchannel

Direct bonding

- Bonding between substrates of same material – no problem of thermal stress from mismatch of expansion coefficient.

Si – Si, Glass – Glass, Polymer – Polymer

Adhesive bonding

- Use of glass layer – sprayed, screen-printed, or sputtered on silicon. Annealing the stack at sealing temperature makes the glass layer to melt and flow. Upon cooling , a strong bond develops.
- Epoxies, UV curable epoxies and photoresists as glue has advantage of low process temperature and can be applied to all kinds of substrate material.

Bonding of two waferscontd.

Eutectic bonding

- A thin gold film, sputtered on silicon wafer.
- Gold-silicon eutectic bonding is achieved at a relatively low temperature of 363°C .

Protocol for Si-Si direct bonding

- Hydration of silicon wafer by immersing in $\text{H}_2\text{O}_2 - \text{H}_2\text{SO}_4$ mixture or boiling HNO_3 , or diluted H_2SO_4 .
- Bonding at elevated temperatures (300°C to 1000°C).
- Annealing (800°C to 1100°C) to improve bond quality.

Bonding of two waferscontd.

Protocol for glass-glass direct bonding

- Cleaning of glass wafer with $\text{H}_2\text{O} - \text{NH}_3 - \text{H}_2\text{O}_2$ solution.
- Removal of moisture at 130°C , followed by thermal bonding at 600°C for 6 – 8 hours.

Metal-metal direct bonding

- Use of high pressure (276 bars) and temperature (920°C) for 4 hours.

Polymer-polymer direct bonding

- For polymers with low surface energy (e.g., PDMS) use of surface treatment with oxygen plasma.

Fluidic interconnects (Press-fit and glued)

Press-fit

- Utilizes elastic forces of coupling parts to seal fluidic access.
- Sealing force is relatively small → suitable for low pressure applications.

Glued

- Adhesives offer good sealing by filling the gap between the external tube and the device opening.
- Surface roughened for better adhesion.
- Polymer glue is used between glass capillary and silicon wafer.

Fluidic interconnectcontd.

- Glass-sealing on metal tube is possible on Kovar tube (alloy made of 29% Ni, 17% Cu, and rest Fe). After fitting Kovar tube in fluidic access, glass beads are placed around them. A carbon fixture around the joint helps annealing the assembly at 1020°C.

Plastic Coupler

- The coupler is a smaller annular material at the tip of the external capillary that is inserted directly into etched opening.
- Thermal annealing allows the plastic to reflow around the opening. Once cooled, hermetic seal results.
- Couplers can offer elastic force to some extent.

Integrated 'O' ring

- Annular area will be etched first by one of the techniques (DRIE say).
- Deposit an oxide (SiO_2) or a nitride (Si_2N_4) layer.
- Silicone rubber will be squeezed into the cavities.
- Fluidic access will be opened from the back side by DRIE.
- Oxide / nitride layer is etched in buffered HF acid and SF_6 plasma.

Biocompatibility - material response

- Generated by the device inside host tissue.
- Caused by diffusion of body-fluid from host tissue into the device.
- Device material swells, develops micro-cracks on the surface → altered mechanical properties, and in some cases leaching.

Biocompatibility - cellular response

- Generated by the host tissue.
- Inflammation at the device-tissue interface, reddening and pain. If the inflammation lasts long and damages local cells → device is cytotoxic.
- Otherwise, chemical signals released by the damaged tissue attract white blood cells.
- A fibrous encapsulation made of foreign body giant cells and macrophages is formed around the device.
- If the encapsulation does not affect the functioning of the device → BIOCOMPATIBLE.
- Tests: *In vitro* (in laboratory glasswares) and *in vivo* (in live animal or human).