Bio- and Drug Delivery MEMS

FINAL EXAM TOPICS

1. Describe the MEMS lithographic procedures and surface and volume machining procedures. Compare as an example the isotropic and anisotropic etching!

2. List the "soft" fabrication possibilities! Describe the difference between "hard" and "soft" fabrication (list some basic materials, procedures)! Where can we use them? What time and spatial resolutions can be achieved with these?

3. Describe the microfluidic principles! Explain why it is difficult to achieve mixing in microfluidics and list some passive or active mixing methods!

4. Describe the electrokinetic phenomena (electrical double layer, electro-osmotic flow, streaming potential, electrophoresis, dielectrophoresis), provide examples of application for each one! Evaluate them critically!

5. What microfluidic base components do you know in addition to the microchannels, from which a complex microfluidic chip can be formed? Make some application examples for each one!

6. List at least 5 of the learned sensor principles and give an example of them (possibly an existing BioMEMS product)! (Figure!)

7. Among the microactuators, list at least 5 actuation principles, including examples (illustrated)!

8. Describe current drug delivery options! What new drug delivery options are available in MEMS technology? Illustrate at least 3 examples!

9. Describe the main methods of Clinical Chemistry (e.g., in the determination of blood and urine components)! Specify the operating principle of flow cytometric methods and what specific diagnostics can be used!

10. Describe the DNS chips! (DNA microarrays) How are the tools built, what is the measurement method? What next-generation sequencing techniques do you know? Please explain at least one in detail!

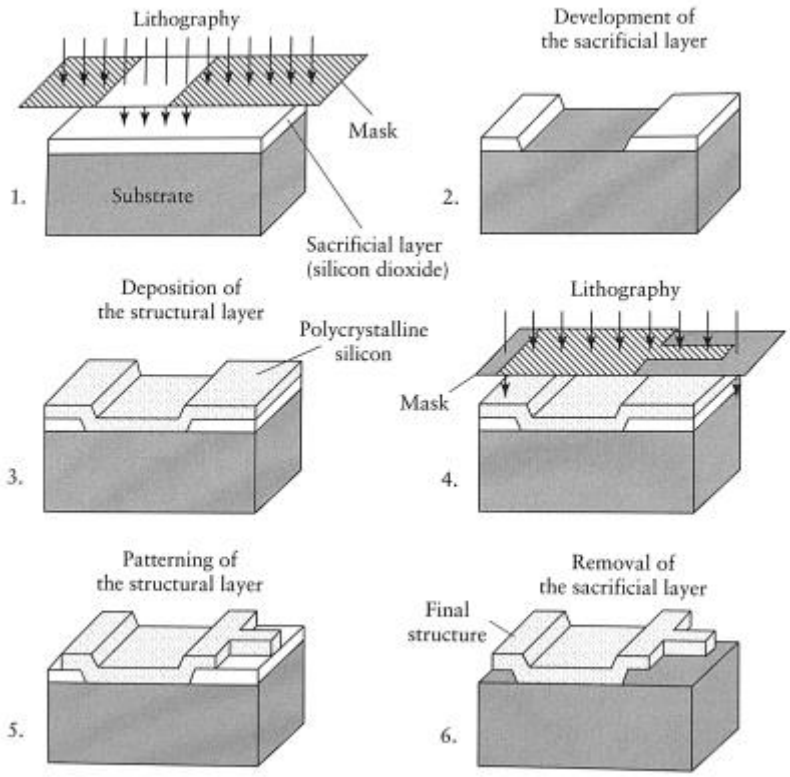
11. Summarize the basic properties of proteins! Explain proteomics and protein chips! List the applicable protein sequencing and identification methods!

12. List at least 4 commercially available BioMEMS devices, operational principles, and evaluate these devices critically! What problems do they provide the solution?

Describe the MEMS lithographic procedures and surface and volume machining procedures. Compare as an example the isotropic and anisotropic etching!

MEMS: **M**icro- **E**lectro-**M**echanical **S**ystems

Basic surface micromachining steps:



- substrate: a silicon wafer, we want to "program" its surface
- sacrificial layer: covers the parts to be protected, later this layer will be destroyed
- photoresist: areas where the resist is removed will ultimately be etched
    - positive: The opaque regions of the mask protect the resist (→ substrate) "positive protects" (e.g., PMMA, DQN)
    - negative: the transparent regions protect the resist when exposed to UV (e.g., SU-8, Kodak KTFR)

Main steps of photolithography:

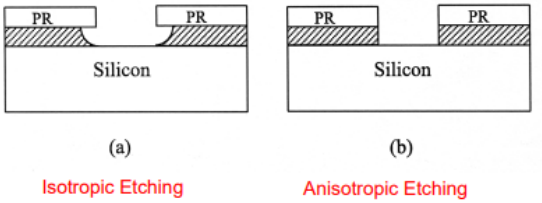
1. Starting point: pure silicon wafer
2. Surface preparation: cleaning process, surface touch, humidity of air → oxidized surface
3. Coating (Spin coating): low speed → covering; high speed → determines thickness
4. Pre-bake (soft bake): gives energy, makes the system a little bit more solid, but polymerization process is not fully finished
5. Alignment: important when there are several layers
6. Exposure: UV exposure at 350-500 nm, finishes the whole polymerization process
7. Development: washes away those parts, which are not polymerized, uses a special solvent
8. Post-Bake (hard bake)
9. Processing using the photoresist as a masking film
10. Stripping
11. Post processing (cleaning)

Etching methods:

- subtractive processes: protect areas that we want to save
  - dry etching: bombards the wafer with energetic ions of noble gases, which knonk atoms from the substrate by transferring momentum
    - glow discharge methods: plasma etching, reactive ion etching, physical sputtering
    - ion beam methods: ion beam milling, reactive ion beam etching, chemical assisted ion beam etching
    - deep reactive ion etching
  - wet etching: a process to chemically remove layers from the surface
- additive processes: cover areas that we want to get rid of

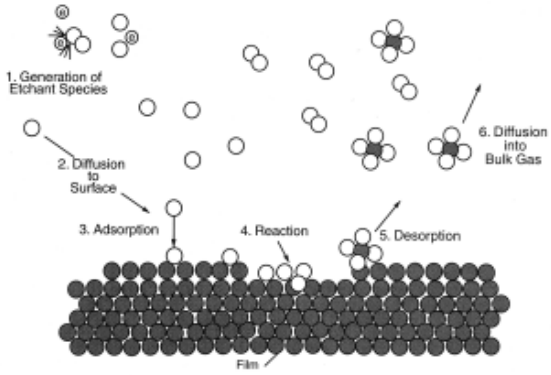
Etching profiles:

- isotropic etching: uniformity in all directions
- anisotropic etching: direction dependent



Plasma etching:

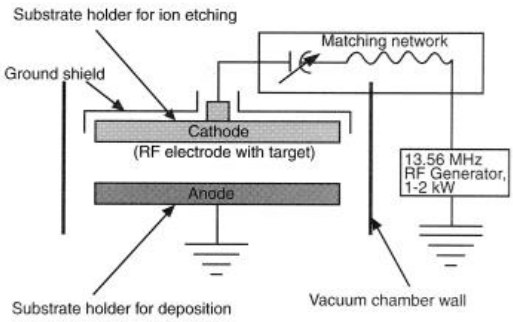
Occurs at relatively lower energy and higher pressure, and is isotropic, selective and less prone to cause damage.



Reactive ion etching:

More middle ground in terms of energy and pressure, with better directionality.

Gases: Argon, Trifluoromethane, Tetrafluoromethane, Oxygen, Sulphur Hexafluoride, Methanol



Physical Sputtering

Relies on physical momentum transfer from higher excitation energies and very low pressures, and results in poor selectivity with anisotropic etching and increased radiation damage. Bombarding a surface with inert ions has an effect related to kinetic energy of the incoming particles.
At energies < 3 eV particles are simply reflected or absorbed.
At surface energies between 4-10 eV some surface sputtering occurs.
At surface energies of 10-5000 eV momentum transfer causes bond breakage and ballistic material ejection across the reactor to the collecting surface. A low pressure and long mean free path are necessary to prevent the material from redepositing.
Implantation (doping) occurs at 10,000 – 20,000 eV.
Sputter yield: the number of atoms removed from the surface per incident ion.

Ion Beam Milling

Relies on physical momentum transfer from higher excitation energies and very low pressures, and results in poor selectivity with anisotropic etching and increased radiation damage.

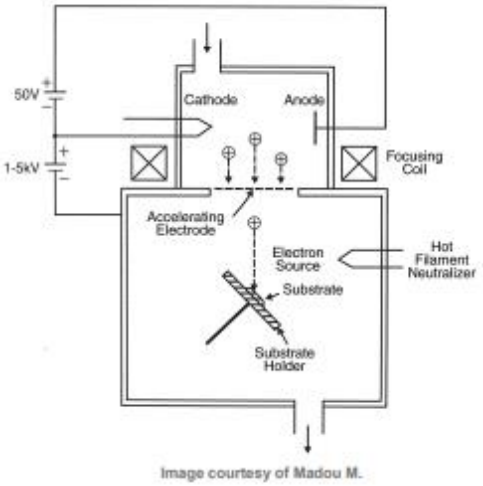
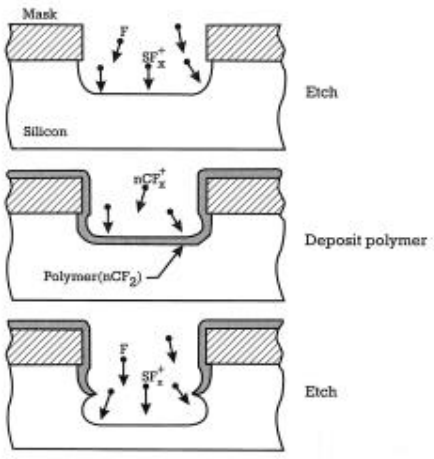


Image courtesy of Madou M.

Deep Reactive Ion Etching

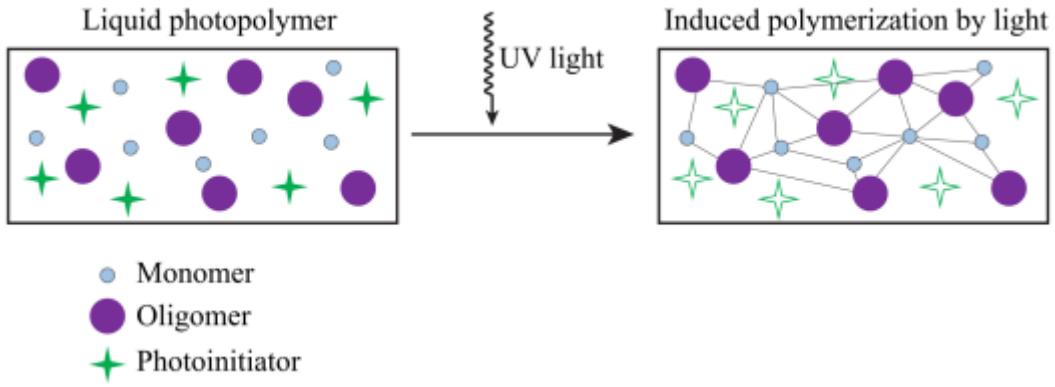
Sulfur hexafluoride is flowed during the etching cycle and octafluorocyclobutane during the sidewall protection cycle.



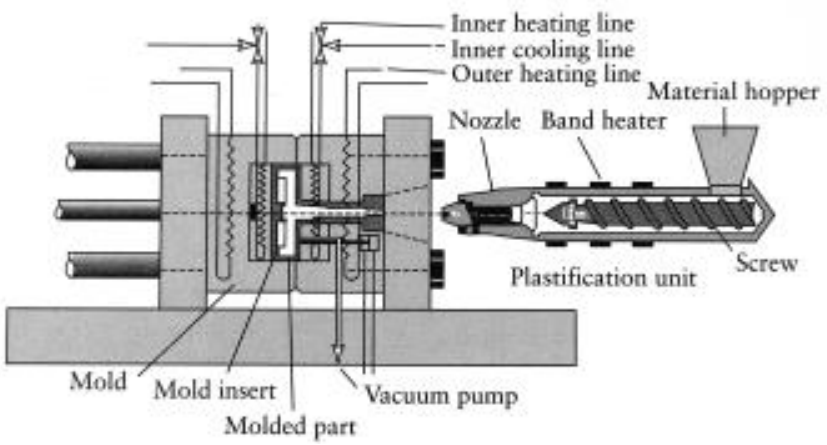
List the "soft" fabrication possibilities! Describe the difference between "hard" and "soft" fabrication (list some basic materials, procedures)! Where can we use them? What time and spatial resolutions can be achieved with these?

Possibilities of soft fabrication:

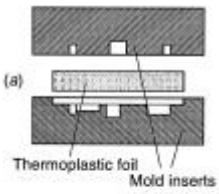
- 3D photopolymerization
  - Based on layer-by-layer assembly
  - Used for rapid production of devices including modelling and prototyping
  - Techniques
    - 3D polyjet printers
    - Stereolithography
    - Microstereolithography
    - Dynamic projection MSL
  - Bioprinting – photocurable hydrogels



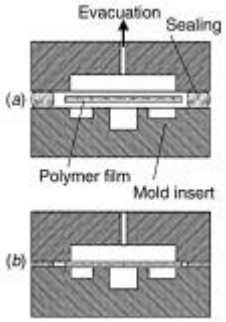
- Injection Molding (Variotherm process)
  - Materials are melted and injected into molds
  - Starting with plastic granules and pushing through heaters we get liquid polymers. Then, the product is created by pushing the liquid polymer into a mold cavity through a nozzle.
  - The mold cavity is the negative shape of the product. In case of microfluidics and MEMS it should be microfabricated for this purpose.
  - The material may be stuck to the surface. → Movable platens can help push it out.
  - Reaction injection molding: mixing different components and then injection.
  - It is also possible to inject metal particles into the mold, with the help of vax.

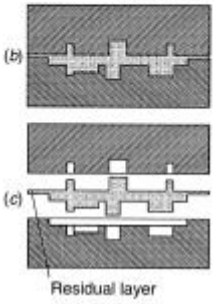


- Hot embossing:
  - Micro-thermoforming technique
  - We have a thermoplastic foil. Using higher temperature we are able to modify this plastic. We have two mold inserts, top and bottom. We put the plastic between these molds applying high pressure.
  - No need for the huge injection molding machine.

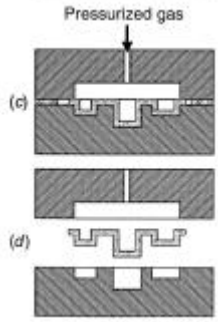


- Thermoforming
  - We have a polymer film and two mold inserts.
  - The upper mold has only a chamber for air.
  - The pressure increases inside the hot mold→ using gas, the film is fitted around the surface.
  - The whole volume of the mold is not filled with the film, only the surface is covered.

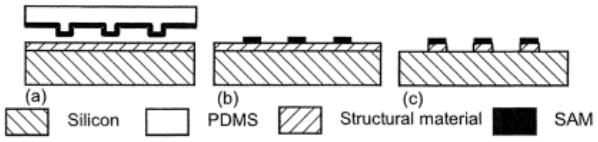




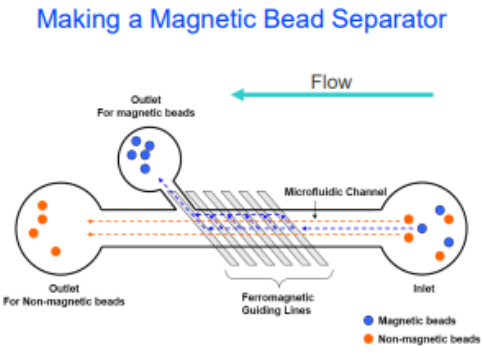
- PDMS stamp

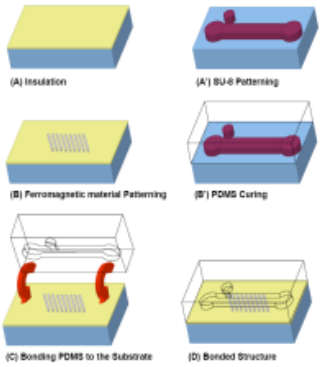


- Microcontact Printing



- PDMS Replica Molding





- Stimuli Responsive Polymers
  - "Smart" polymeric materials exhibit significant changes in their characteristics with small changes in their environment.
  - These external stimuli include pH, Ca, Mg, organic solvents, temperature, magnetic field, electrical potential, IR, UV radiation.
  - Some materials respond to dual stimuli such as Ca and PEG, Ca and temperature, Ca and acetonitrile, pH and temperature, light and temperature
  - Electroactive polymers (EAPs) respond to electrical stimulation.
- Hydrogel Design
  - Design considerations:
    - Hydrogels may be selectively polymerized by using UV light, a collimating microscope and photolithography masks. An energy level of 40 mW/cm_2 can induce polymerization.
    - Biocompability
    - Vascularization
    - Degradation

- Network structure and properties
  - Physical structure
  - Equilibrium swelling
  - Rubber elasticity
- Applied in tissue engineering

Difference between "HARD" and "SOFT" fabrication

Advantages of soft fabrication

- Lower cost than traditional photolithography in mass production
- Well-suited for applications in biotechnology
- Well-suited for applications in plastic electronics
- Well-suited for applications involving large or nonplanar surfaces.
- More patter-transferring methods than traditional lithography techniques
- Does not need a photo-reactive surface to create a nanostructure
- Smaller details than photolithography in laboratory settings (~30 nm vs ~100 nm). The resolution depends on the mask used and can reach 6 nm.

Soft fabrication possibilities:

- Biomaterials: in contact with a living system: implants, transport or containment, process function
- Fast prototyping – PDMS
- Self-assembly

	Materials	Procedures	Applications	Resolution
Hard	Silicon Gallium, Arsenic Metals Glasses Quartz, Sapphire, Ceramics	Lithography Etching Thin film deposition	Sensors Actuators Microfluidics	Photolithography – 100 nm Direct write E-beam – 5 nm
Soft	Polymers Composites Epoxy resins Rubbers	Molding Casting Laser ablation Sharp stylus	Implants Diagnostics Microfluidics	Injection Molding – 10 nm

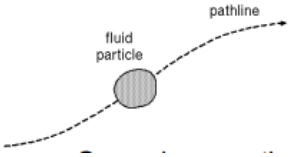
Method	Resolution
Injection molding	10 nm
Embossing (imprinting)	25 nm
Cast molding	50 nm
Laser ablation	70 nm
Micromachining with a sharp stylus	100 nm
Laser-induced deposition	1 um
Electrochemical micromachining	1 um
Silver halide photography	5 um
Pad printing	20 um
Screen printing	20 um
Ink-jet printing	50 um
Electrophotography	50 um
Stereolithography	100 um
Soft Lithography	
Microcontact printing	35 nm
Replica molding	30 nm
Microtransfer molding	1 um
Micromolding in capillaries	1 um
Solvent-assisted micromolding	60 nm

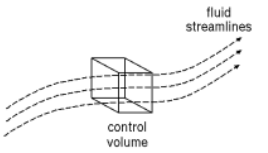
Describe the microfluidic principles! Explain why it is difficult to achieve mixing in microfluidics and list some passive or active mixing methods!

Microfluidic principles

- Main fluidic areas:
  - Continuous microfluidics
    - Sample material is injected into a stream as a plug, with interspaced amounts of buffer solution.
    - Pressure-driven flow is commonly used to transport the plugs through a network of interconnecting channels where reagents may be added and mixed, allowing reactions to occur.
    - Single-phase CMF (just one liquid)
    - Multi-phase CMF (multiple liquids/liquid gas)
    - Darcy based CMF (nano-fluidics)
  - Digital microfluidics
    - Droplets are moving on hydrophobic surface driven by electric fields.
- Fluids
  - Have volume, but no shape
  - Any substance that deforms continuously under the application of shear
  - Behaves as continuum
  - Sticks to surface (no-slip condition)
- Newton's second law → Navier-Stokes equations
  - Describe the motion of viscous fluid substances
  - Mathematically express momentum balance and conservation of mass for Newtonian fluids
- Incompressible laminar flow
- Squeeze-film in MEMS
- Type of fluids
  - Newtonian: A fluid in which the viscous stresses arising from its flow are at every point linearly correlated to the local strain rate – the rate of change of its deformation over time. Stresses are proportional to the rate of change of the fluid's velocity vector.
  - Non-Newtonian: Everything which is no Newtonian.
- Reynolds number: ratio of inertial forces to viscous forces.
- Types of flow
  - Laminar
    - Low Reynolds number – viscous forces are dominant
    - Smooth, constant motion
    - Fluid particles move in separate laminas (layers, high friction between layers)
  - Turbulent
    - High Reynolds number – inertial forces are dominant
    - Vortices, eddies, chaotic behaviour
    - Fluid particles move along irregular paths
- Properties of fluid
  - Density, shear viscosity, kinematic viscosity, surface tension, thermal variation, thermal diffusivity, compressibility, coefficient of thermal expansion

- Lagrangian vs Eulerian description:
    - A fluid flow can be thought of as being comprised of a large number of finite sized fluid particles which have mass, momentum, internal energy, and other propertied. Mathematical laws can then be written for each fluid particle. This is the Lagrangian description of fluid motion.
    - In the Eulerian description of fluid motion, we consider how flow properties change at a fluid element that is fixed in space and time (x,y,z,t), rather than following individual fluid particles.





- Main flow profiles:
    - Uniform: Velocity vector is homogenous and unidirectional
    - Parabolic: Velocity is higher in the middle of the channel
    - Shear: Velocity is highest next to the moving wall.
- Streamline: a line that is everywhere tangent to the velocity field. In steady flows, the streamline of the same as the path line, the line traced out by a given particle as it flows from one point to another.
- Governing equations:
    - Conservation of mass
    - Conservation of momentum (Newton's second law): the change of momentum equals the sum of forces on a fluid particle.
    - Conservation of energy (First law of thermodynamics): rate of change of energy equals the sum of rate of heat addition to and work done on fluid particle.
- Conversion of mass →continuity equation
- Fluid element is a volume stationary in space, and a fluid particle is a volume of fluid moving with the flow.

Diffusion – difficult to achieve mixing in microfluidics

Microfluidics is not very good in flow mixing, because diffusion is the only possibility which we have. For example, there are two components, comp A and comp B, and there are two inlet channels. In this case there is one surface and on the surface, diffusion takes place.

To increase the efficiency you need to multiply the surface area. → increase the number of streamlines of A and B, putting them close to each other → this case we get several surfaces. It is easier to solve this problem in 3D devices.

Active mixing:

Mixing is enhanced by the application of some form of external energy disturbance.

Examples: using ultrasound waves, heating up the fluid to get higher diffusivity

Passive mixing:

Achieved by altering the structure or configuration of fluid channels. This type of mixing is incorporated into the system during fabrication and is not externally controlled by users.

Example: Fishbone system (molecular mixer)

Describe the electrokinetic phenomena (electrical double layer, electro-osmotic flow, streaming potential, electrophoresis, dielectrophoresis), provide examples of application for each one! Evaluate them critically!

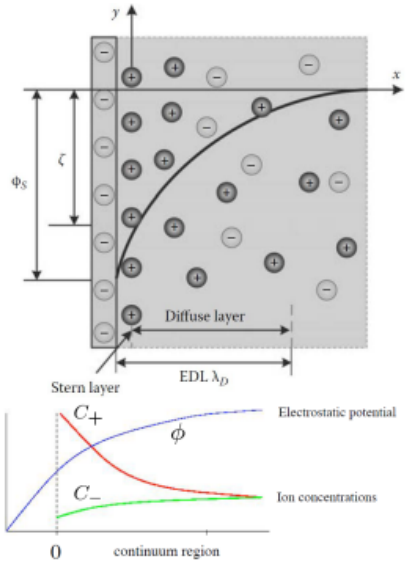
Electrical double layer

Most solid surfaces tend to gain surface charges when they are brought into contact with ionic aqueous solutions.

The origin of the surface charge mainly arises from the adsorption or dissociation of chemical groups.

The electrostatic interaction between the charged surface and the surrounding ions attracts counterions and repels co-ions from the charged surface.

As a result, a thin layer predominantly occupied with more counterions is formed in the vicinity of the charged surface, referred to as the EDL.



Example: if we charge the surface with negative ions, many positive particles will get close to the surface due to the electric charge. The probability of this is exponential, so close to the walls there are more oppositely charged particles than further away from the surface.

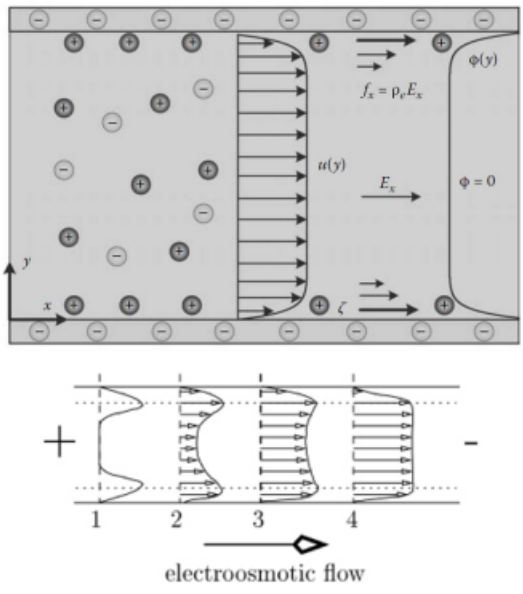
All materials have different characteristics of it.

Ions within the stern layer are immobilized due to a very strong electrostatic force; ions within the diffuse layer are free to move. (this is why the surface becomes a conductive surface) The electric potential arising from the net charge within the diffuse layer obeys the classical Poisson equation. The length of this EDL can be described by the Poisson-Boltzmann equation.

Electro-osmotic flow

Electro-osmosis = fluid slip across the double layer, as an electric field pushes on the screening cloud.

When an external electric field is applied parallel to a stationary charged surface, the excessive counterions within the EDL of the charged surface migrate toward the oppositely charged electrode, dragging the viscous fluid with them.



The induced flow motion arising from the electrostatic interaction between the net charge within the EDL and the applied electric field is called electro-osmosis, also called electroosmotic flow (EOF).

If $u = \mu_e \nabla\phi$, and $\nabla^2\phi = 0$.

Electro-osmosic slip:

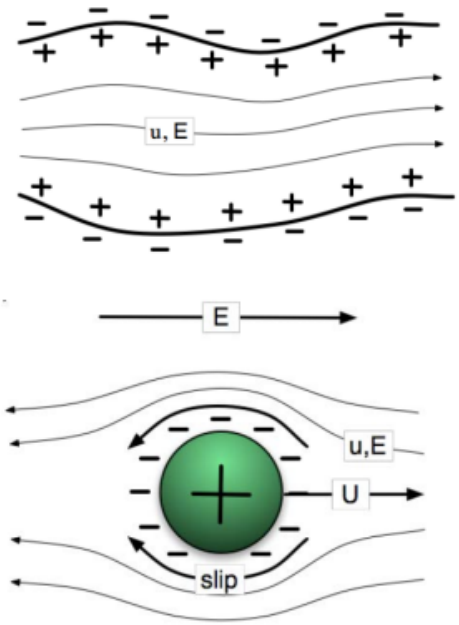
$$u_s = -\mu_e E$$

Electrophoretic mobility:

$$\mu_e = \frac{\varepsilon\zeta}{\eta}$$

Zeta potential

$$\zeta = \Delta\phi = \frac{q}{c_D}$$



Electro-osmotic flow can be used for membrane-based separation, transporting ions through the membrane.

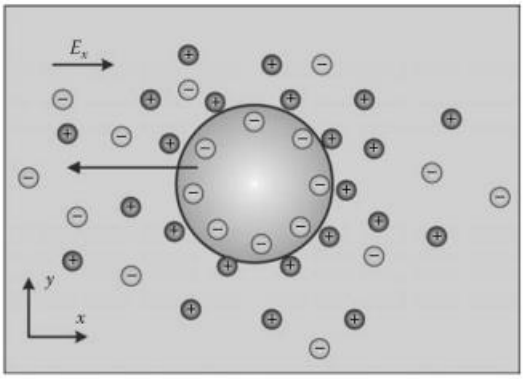
Streaming potential

- Opposite of the electro-osmotic flow
- Due to EDL there are more counter-ions in the channel
- Fluid is pressure driven → stern layer of EDL moves → moving charged particles = current
- Surplus of counter ions in the channel is replaced with a more neutral fluid → potential
- Can be used to track aggregation of particles.

Electrophoresis

Electrophoresis refers to the migration of charged particles suspended in an aqueous solution subjected to an external electric field.

The charged surface is stationary in EOF; however, it becomes mobile in electrophoresis.



Electrophoresis = particle motion due to electro-osmosis

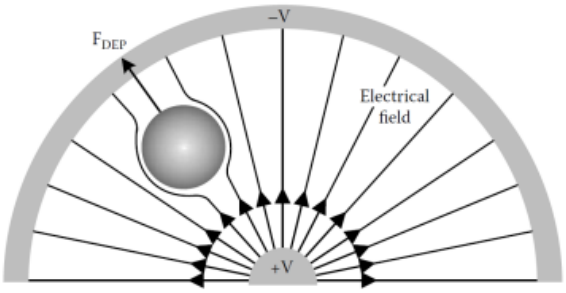
- Promoting factors: voltage, current, high surface charge, low mass, molecular dissociation, pH
- Retarding factors: physical resistance, viscosity, interactions, low surface charge, high mass, molecular association, non-spherical shape
- Can be used for separation of particles on charge & mass
- Electro osmotic pumps

Dielectrophoresis

Dielectrophoresis refers to the motion of polarizable particles immersed in an aqueous solution subjected to a spatially nonuniform electric field.

The ratio of the polarizability of particles to that of the electrolyte solution determines the direction of the DEP force.

A positive (negative) dielectrophoresis refers to the DEP force directed toward (away from) the region with a higher electric field.



AC electric fields can selectively manipulate particles, separation applications

What microfluidic base components do you know in addition to the microchannels, from which a complex microfluidic chip can be formed? Make some application examples for each one!

- Micropumps: active drug delivery systems
- Microvalves: active drug delivery systems, lab-on-a-chip regulation of fluid transport
- Microturbines
- Microsensors: surgical instruments, flow-, pressure-sensors
- Microfilters: separation of particles, diagnostics
- Micromixers: reaction catalyzation/control
- Microreactors: production of chemicals (precisely controlled)
- Microdispensers
- Microseparators: isolating paritcles, separating particles based on certain criteria

List at least 5 of the learned sensor principles and give an example of them (possibly an existing BioMEMS product)! (Figure!)

Sensor: measures information from the environment (e.g. blood analyte, or measurand) and provides an electrical signal in response.

Sensors may be classified in various ways:

- Measurand – temperature, pressure, flow, etc.
- Transduction (physical and chemical effects) – SAW, ion selective FETs, optrodes etc.
- Materials – resistive, piezoelectric, magnetic, permeable membranes, etc.
- Technology – MEMS, bioMEMS, plasmon resonance, CMOS imaging, charge coupled devices etc.
- Energy requirement – active or passive
- Applications – industrial, automative, aviation, consumer electronics, biomedical etc.

Common Microsensor Types

- Thermal sensors – measuring changes in temperature
    - Thermomechanical
    - Thermoresistive
    - Thermocouples
- Mechanical sensors – properties of strain, force and displacement
    - Piezoresistive – strain in a semiconductor changes resistivity
    - Piezoelectric – strain in a piezoelectric crystal causes a potential
    - Capacitive – electrostatic, parallel plates and displacement
    - Resonant – microfabricated beams and bridges
- Chemical sensors – interaction with solids, liquids, and gases
- Radiant sensors – ionizing radiation and visible, infrared or UV light
- Biosensors – measurement of biological analytes

<u>Piezoelectric sensors</u>

- Work based on the piezoelectric effect.
- Direct effect: When mechanical stress is applied a voltage is generated across the material.
- Converse effect: Application of an electrical field creates mechanical deformation in the crystal.
- Polling: Random domains are aligned in a strong electric field at an elevated temperature.
- Three modes of operation depending on how the piezoelectric material is cut: transverse, longitudinal, shear.
- Amplifiers are needed to detect the small voltage.
- Mechanical strain is proportional to the electric field and the displacement (or charge density) is proportional to the stress.
- Piezoelectric materials: crystals, quartz, ceramics, polyvinyiledene fluoride (PVDF, polymer)
- Piezoelectric devices have to be fabricated
    - Depostion of piezoelectric thin films.
    - Direct bulk micromachining.
- Application:
    - Mechanical transducer: We have a cantilever, in the base of the cantilever there is a piezoelectric crystal. As the cantilever moves, the piezoelectric crystal is strained that means there is voltage difference between the two ends of the crystal. This can be amplified and measured. The effect is proportional to the movements of the cantilever.

- Resonator transducer: Using surface acoustic waves the voltage changes can be measured. If this shifts, it means the surface has changed and we can calibrate depending on biomolecules or sensor surface.

Thermosensors

- Resistors: Resistance is temperature dependent
  - Platinum resistors
    - Linear, stable, reproducible
    - Material property dependency on temperature
- Thermocouple: (e.g. Type K)
  - Potentiometric devices fabricated by joining of two different metals forming a sensing junction.
  - Based on thermoelectric Seebeck effect in which a temperature difference in a conductor or a semiconductor creates an electric voltage.
  - The two metal structures are a little bit different, and this gives an electric dependent conductive change on the two metal surface.
  - Simple setup but requires very well insulated reference temperature.
- Thermistor: a semiconductor device made of materials whose resistance varies as a function of temperature.
- Thermodiode and Thermotransistor
  - When a p-n diode is operated in a constant current (IO) circuit, the forward voltage (Vout) is directly proportional to the absolute temperature (PTAT).

Microforce Measurements

- Considerations:
  - Contact force feedback is essential for microassembly.
  - Forces may be in the micro-newton range.
  - Micromanipulation (handling micro-scale objects) – e.g. cells or capillaries
  - Other micro-components are easily destroyed – e.g. microgrippers.
  - Alignment of micro-optical systems.
- Force sensing methods:
  - Strain gauge-based fore sensor
  - Piezoresistive force sensor
  - Capacitive force sensor
  - Piezomagnetic force sensor
- Calculating Young's modulus:

Stress

$$\sigma = \frac{F}{A}$$

Where…
σ stress (Mpa),
F is the force (N),
A is the cross sectional area (mm^2)

Strain

$$\epsilon = \frac{(L - L_o}{L_o}$$

Where…
ϵ is the strain,
L is the stretched length,
L_o is the initial length (mm)

Young's Modulus (Stress/Strain)

$$E = \sigma/\epsilon$$

- Application:
    - Piezoresistive Sensor:
        - Most common technique to measure microforce.
        - When a metal or semiconductor material is under stress, its resistance will change proportionally to its deformation.
        - Wheatstone bridge can be used to translate variation in resistance to voltage.
    - Capacitive force sensor:
        - Functions by measuring force by changes in the distance between plates.
        - Able to measure normal and shear stress.
        - Range: mN to pN
        - RC circuits may account for up to 30% of sensors.
        - Signals are obtained by capacitance fo frequency conversion (oscillator), switched capacitor or capacitive AC bridge circuits.
    - Piezomagnetic force sensor:
        - Magnetoelastic effect – when a ferromagnetic material subjects to mechanical stress, its internal strain leads to the changes in permeability.
        - Dynamic and static force measurements.
        - Does not need to be glued to the surface.

<u>Flow sensors</u>

- Measurement of gas and liquid flow rates.
- May be integrated with microfluidics.
- Useful for blood and urine flow, respiratory monitoring, and drug delivery devices.
- Advantages of high sensitivity, accuracy and precision, low power consumption and small size.
- Broadly categorized as thermal (thermal exchange) and non-thermal flow sensors.
- Application:
    - Thermal flow sensors:
        - Hot wire or hot element anemometers
            - Based on convective heat exchange taking place when the fluid flow passes over the sensing element (hot body)
            - Operate in constant temperature mode or in constant current mode.
        - Calorimetric sensors
            - Based on the monitoring of the asymmetry of temperature profile around the hot body which is modulated by the fluid flow.
    - Non-thermal flow sensors:
        - Cantilever type flow sensors: Measuring the drag force on a cantilever beam
        - Differential pressure-based flow sensors
            - When a fluid flow passes through a duct, or over a surface, it produces a pressure drop depending on the mean velocity of the fluid.
        - Electromagnetic
        - Laser Doppler flowmeter
            - The phenomenon is due to the interaction between an electromagnetic or acoustic wave and a moving object: the wave is reflected back showing a frequency different from the incident one.
        - Lift-force and drag flow sensors
            - Based on the force acting on a body located in a fluid flow.
        - Micromotor
            - Rotating turbine
        - Resonating flow sensors
            - Temperature effects resonance frequency of a vibrating membrane.

Biosensors:

- Application of previous sensor principles (either thermal, force or any other principles) to turn biochemical reaction into measurable electric signal.
- A biological or biologically derived sensitive recognition element usually is immobilized on a transducer to measure one or more analytes.
- Analytes:
    - Biological elements (smaller or larger elements, cells)
    - Very wide range in terms of type and size.
    - Depending on chemical composition different approaches are necessary.
- Biorecognition element (the size of biosensor)
    - Specifically selective for the desired analyte.
- Transducer
    - Biorecognition elements have to be integrated by the transducer.
    - Always dependent on measured properties.

Quartz Crystal Microbalance

- QCM measures a mass per unit area by measuring the change in frequency.
- The resonant frequency is disturbed by small mass change due to the deposition of thin film or molecules at the surface of resonator.
- Can be operated under vacuum or liquid
- Application:
    - Biotechnology
        - Interactions of DNA and RNA
        - Specific recognition of protein ligands by immobilized receptors, immunological reactions
        - Detection of virus, bacteria, mammalian cells.
        - Adhesion of cells, liposomes and proteins
        - Biocompatibility of surfaces
        - Formation and prevention of biofilm formation
    - Functionalized surfaces
    - Thin film formation:
    - Surfactant research
    - Drug research

Surface Acoustic Wave Devices

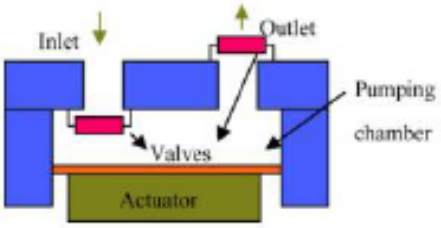
- A travelling wave on the crystal structure.
- Crystal structure is moving on piezoelectric substrate or thin film using interdigitated electrodes.
- The two integrated electrode sets as capacitors:
    - One of them creates the surface acoustic wave.
    - The other one records the wave.
- If anything is changing on the surface, then frequency shifts
- Very selective and sensitive.

Among the microactuators, list at least 5 actuation principles, including examples (illustrated)!

These techniques are based on some sort of pumping mechanism. Depending on what forces we use we can categorize.

Mechanical displacement micropump

Plug in electric current or voltage and change the voltage into some kind of mechanical displacement. This mechanical displacement will affect propelling the liquid or moving the liquid from one side to the other side.



Parts:

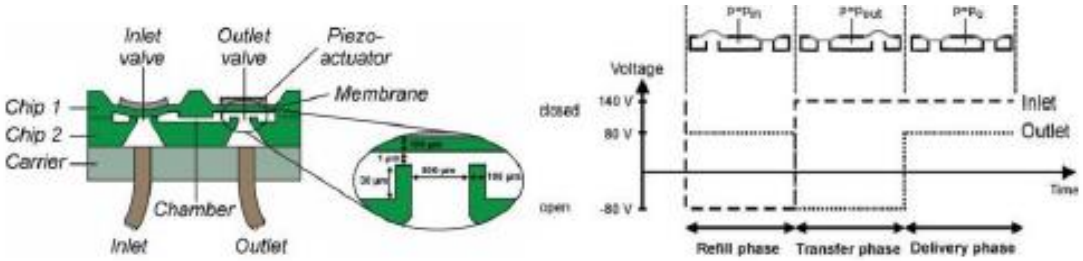
- Inlet, Outler
- Chamber
- Valves: Help not to let the liquid flow backwards.

Implantable Microport System

- Control unit
- Battery
- Micropump
- Reservoir: Relatively low cc → Due to any mistakes it van let out higher doses.
- Port septum: to refill
- Gas buffer: Can fill up the whole volume not to let vacuum develop.

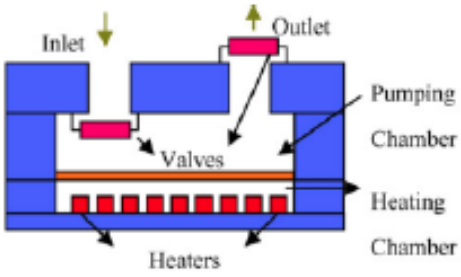
Piezoelectric pump

- If voltage difference is applied, it changes its shape.
- Filters and valves are required. (Any unwanted particles can break the piezoelectric material)
- Depending in the frequency, the flow rate can be set.



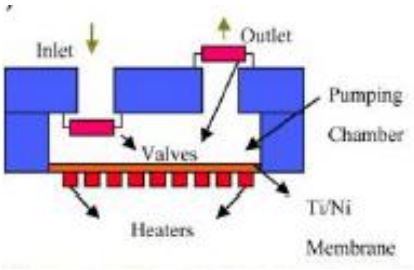
Thermopneumatic micropump

- Liquid/gas is heated up → volume increases → the boundary plate bends → pumping



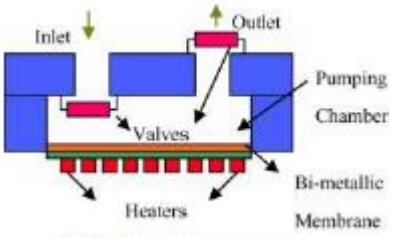
SMA pump

- Shape memory Ti/Ni alloy
- It has a crystal structure that contains two different phases. If it gets into a certain shape it can preserve its original shape (which was originally molded.
- It can be deformed when cold, but returns to its pre-deformed shape when heated.



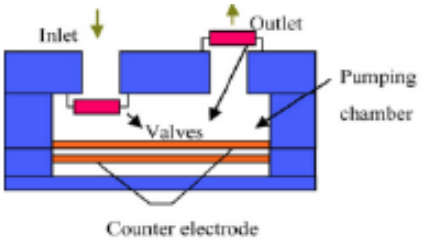
Bimetallic pump

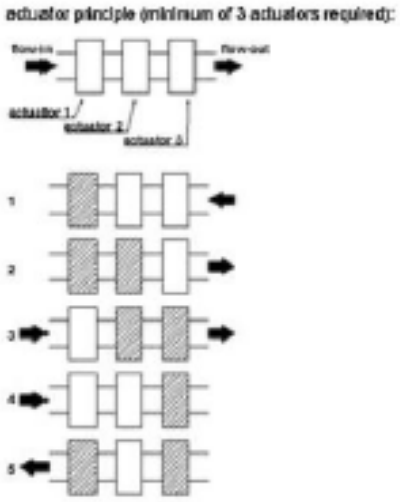
- Bonding of two dissimilar materials with different coefficients of thermal expansion.
- If we heat them up or cool down, they will bend because one of the metals has a higher expansion coefficient than the other one. Since they are bonded together the other one will deflect and it will result in chamber volume changes.



Ionic-conductive polymer film

- If we apply different voltage for counter electrodes, the upper electrode will change its shape →the volume of the chamber increases.
- Electrostatic force moves the plates closer or further away from each other.
- Peristaltic configuration
- 3 electrostatic pumps, one after the other

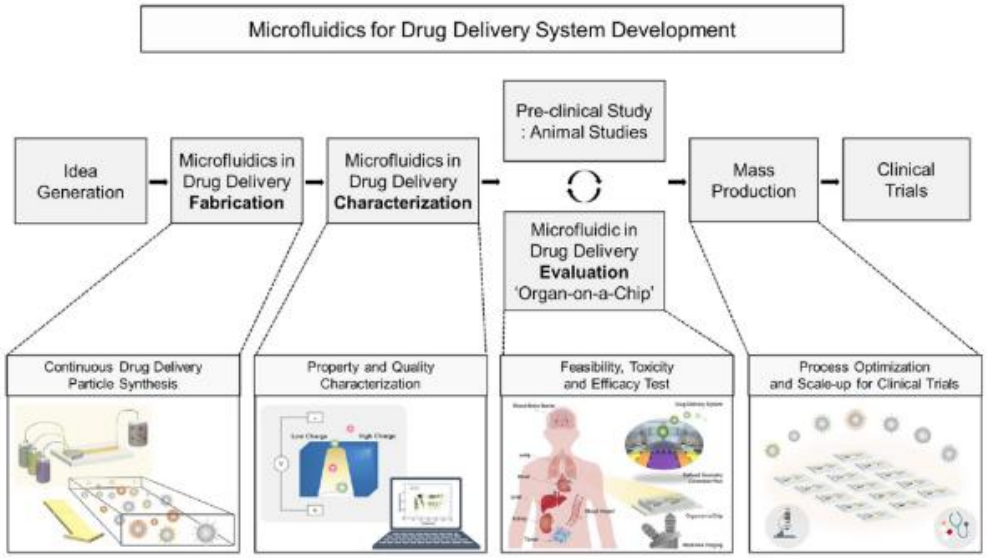


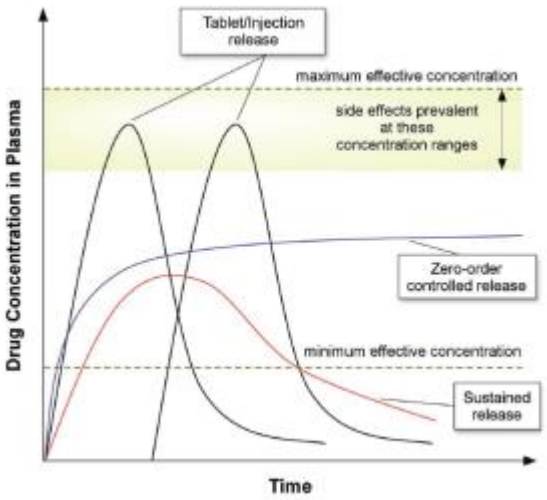


Describe current drug delivery options! What new drug delivery options are available in MEMS technology? Illustrate at least 3 examples!

Routes of Administration:

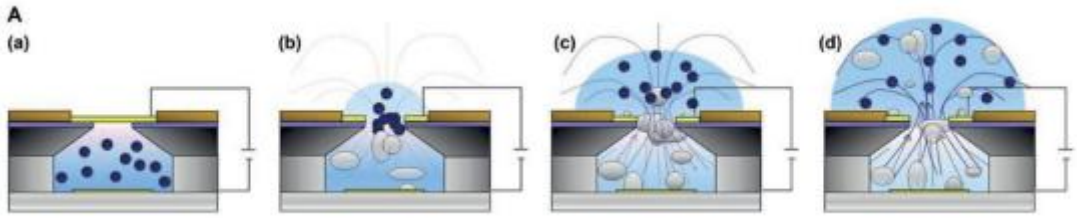
- Oral delivery
- Surgical implantation
- Buccal delivery
- Ocular delivery
- Pulmonary delivery
- Transdermal delivery
- Systemic Delivery
- Vaginal delivery





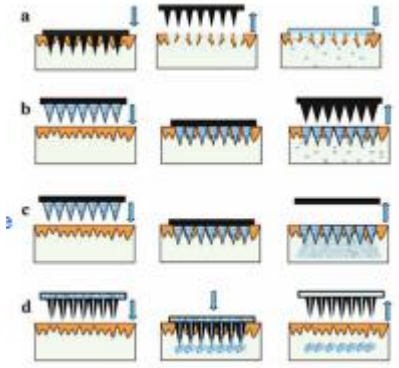
Ultimatr goal: controlled release of drugs:

Reservoir Devices for Drug Delivery



- An electrochemically driven microfluidic drug delivery device.
- The electric potential is applied between top and bottom electrodes.
- Two main electrochemical reactions occur: dissolution of the gold membrane and electrolysis of water resulting in gas release.
- The generated microbubbles propel drug solution out.
- The reaction continues until fluid transport stops.

Transdermal Drug Delivery

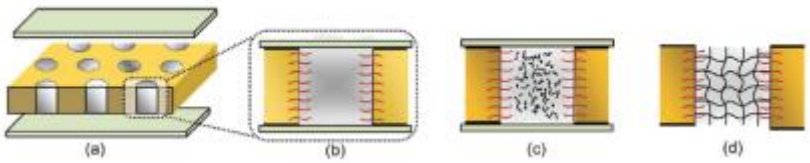


- Solid needle making transient micropores.
- Drug coated needles.
- Soluble polymeric/carbohydrate microneedles containing drug that dissolve in skin.
- Hollow needle.

3D Additive Manufacturing

- Motivations:
  - Product complexity, personalization, on-demand, onsite fabrication, potential for low-cost production
- Complex geometries
  - Potential for various tablet infills and controlled release with small holes.
- Expect close scrutiny by the FDA.

Drug Carrier Membrane



- Pore-filling functionalization via in situ photopolymerization during different stages
  - Filling and equilibration of the membrane.
  - During equilibration with reaction mixtures.
  - During UV initiated in situ crosslinking polymerization
  - After complete reaction toward hydrogel pore-filled composite membrane.

Priston Drive Capsule Delivery

RF & SMA Actuator

Hydrodynamic focusing

Describe the main methods of Clinical Chemistry (e.g., in the determination of blood and urine components)! Specify the operating principle of flow cytometric methods and what specific diagnostics can be used!

Depending on the size of the molecules we are looking at, different tools exist for analysis:

- Chemistries & Immunology, Hematology, Microbiology, Urinalysis, Anticoagulation, Blood gas, Cytology, Pathology

Chemisitries & Immunology use Antibodies for most of the applications

Fluorophore: fluorescent chemical compound that may re-emit light upon light excitation.

- Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with several π bonds.
- Generally covalently bonded to a macromolecule, serving as a marker for affine or bioactive reagents.
- Fluorophores are notably used to stain tissues, cells, or materials in a variety of analytical methods.
- Main idea: use fluorophore linked antibodies and measure how many antibodies are left in the liquid

<u>ELISA</u>

- Enzyme-linked Immunosorbent Assay
- A biomolecular technique that utilizes the specificity of an antibody as well as the sensitivity of enzyme assays, to detect and quantify molecules such as hormones, peptides, anitbodies and proteins.
- Uses:
    - Identification of cancer biomarkers for early detectionof cancer.
    - Drug screening and concentrations in patients undergoing treatment.
    - Pregnancy screening
    - Detection of platelet antibodies
    - Virus detection
- Methodologies:
    - Direct ELISA
    - Indirect ELISA
    - Sandwich ELISA
    - Competitive ELISA

<u>Chemiluminescence Immunoassays (CLIA)</u>

- Rapid and accurate diagnosis of autoimmune disease
- The label is a luminescent molecule.
- Chemiluminescent methods:
    - Direct – using luminophore markers.
    - Indirect – using enzyme markers.
    - Either method may be competitive or non-competitive.

Nephelometry

- Based on the scattering or absorption of light by solid or colloidal particles suspended in solution.
- Used in immunology to determine the levels of several blood plasma proteins.
- Depending on the size of particles in the solution we can measure the cc of the particles by either the light intensity change going through the solution or the scattered light.
- It is useful when the size of antigen is low. Combined with antibodies (even more antibodies can attach to the same antigen) → creating large molecules → the solution becomes colloid which scatters light.

Lipoprotein analysis

- Separation by electrophoresis
  - Separating plasma from the whole blood
  - Separation based on net surface charge and that will create different bands.
  - Based on band size type we can analyse high density LP, low density LP and very low-density LP
- Separation by ultracentrifugation
  - Higher centrifugation force will separate the different densities.
  - Add specific solution bands corresponding to certain classes.
  - Only the density of particle is measured.

Spectrophotometric assays

- Method for cc measurement.
- Measures the colour change of the liquid quantitatively, but it does not have to be an Ab + Ag reaction.

Protein Electrophoresis

- A test that measures specific proteins in the blood.
- It separates proteins in the blood based on their electrical charge. The protein electrophoresis test is often used to find abnormal substances.
- Electrophoresis is a laboratory technique in which the blood is applied to either gel matrix, or into liquid in a capillary tube. Then, it is exposed to an electric current to separate the serum protein components into major fractions by size and electrical charge.

Antinuclear antibodies (ANA):

- Normally antibodies, produced by the white blood cells recognize and combat infectious organisms in the body.
- ANAs are produced by a person's immune system, and mistakeny directed towards normal, naturally occurring proteins in our bodies.
- By itself, a positive ANA test does not indicate the presence of an autoimmune disease or the need for therapy.
- Diseases include lupus, scleroderma, Sjörgen's syndrome…

Direct & Indirect Immunofluorescence

- Direct IF: uses a fluorophore-conjugated antibody to stain the target protein.
- Indirect IF: involves first binding the primary antibody to the target, then detecting the primary antibody using a conjugated secondary antibody.

Complete Blood Count

- Platelets, RBC and WBC can be distinguished by volume and size.
- Sensors count and identify the cells in the sample using electrical impedance. Impedance-based cell counting: cells are suspended in a fluid carrying an electric current, and as they pass through a small opening (an aperture), they cause a decrease in current because of their poor electrical conductivity. The amplitude of the voltage pulse generated as a cell crosses the aperture correlates with the amount of fluid displaced by the cell, and thus the cell's volume, while the total number of pulses correlates with the number of cells in the sample. The distribution of cell volumes is plotted on a histogram, and by setting volume thresholds based on the typical sizes of each type of cell, the different cell populations can be identified and counted.

Basic Flow Cytometry

- Cells travel in a single-cell stream.
- The stream is illuminated by light/laser.
- Measuring the transmitted light and the scattered light:
    - Forward scatter: amount of transmitted light – cell volume
    - Side scatter: amount of 90° scattered light – higher density internal structures induce higher side scatter
- Counting and identifying different cell types.

Flow cytometry with cell markers:

- Add fluorescent labelled Ab. to the sample (which is usually blood), mix it. (Use more fluo. labels)
- These will bind to the right target on the surface.
- Differentiate cell types: Tumour cells which have similar size as WBC, but tumour cells can be tagged by fluo. labels.
- Use the same Cytometry system but measure also the colour of the light. Also, based on the emitted light we can classify the different type of cells
- Flow Cytometry setup is quite complicated because the incident and scattered light are separated with different mirrors to recognise different fluo colour lights.
- Have to be avoid the overlapping light wave range.

Gram stain:

- Method to recognise microorganisms.
- There are a series of stains, which are able to attach to the cell wall, to the membrane of the microbes.
- Gram staining will stain certain bacteria usually.

Urinalysis:

- Concentrated urine sediment.
- Phase contract microscopy.

Describe the DNS chips! (DNA microarrays) How are the tools built, what is the measurement method? What next-generation sequencing techniques do you know? Please explain at least one in detail!

DNA microarrays

- Manufactured through a combination of photolithography and combinatorial chemistry.
- These microarrays are based on Si technology.
- Structure:
    - Single stranded DNA fragments (25-30 bases long) are immobilized on the surface of silicon sheet.
    - 11 um squares are filled with identical DNA fragments (A total of around 500 000 locations, for different DNA fragments, millions of DNA for same square).
    - Array of squares (containing different fragments) makes up the chip.
- Goal: To measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome.
- The basic working assumption: the concentration of the mRNA molecules in a cell define its biological state
- Form DNA spot pf a specific DNA sequence (i.e. probes or reporters), which can be a short section of a gene that are used to hybridize a cDNA or cRNA sample (i.e. target).
- The detection methods of probe-target hybridization: florophore-, chemiluminescence, or silver-labelled targets.
- Allow parallel analysis of thounsands of genes on a single chip.
- Provide multiple order-of-magnitude increases in throughput.
- Save time and materials compared to Northern blot, RT-PCR, and Ribonuclease Protection Assays.
- Applications:
    - Functional genomics, Basic life science research, Drug discovery and development, Clinical diagnostics and prognostics, Gene discovery, Sequencing, Mapping and gene expression, Single nucleotide polymorphism

Microfabrication of DNA Chips

- Step 1: Make gene probes
    - Using conventional techniques such as polymerase chain reaction and biochemical synthesis, strands of identified DNA are made and purified. A variety of probes are available from commercial sources, many of which also offer custom production services.
- Step 2: Micro- or nano-lithography
    - To turn glass and plastic wafers into receptacles for the DNA probes.
- Step 3: Variety of processes ranging from electrophoretic bonding to robotic deposition to adhere genetic material to the substrate. Cleanroom conditions to attain the degree of contamination control needed during the deposition process.
- Step 4: DNA chips enable researchers to identify the components of probes deposited on the chips.

Measurement method:

- Sample preparation
- Separate DNA strands (measurands)
- Single stranded DNA is spliced into segments with the needed length (25-30 bases for a DNA micro-array)
- Each segment needs to have an indicator (e.g. fluorescent dye at the end of the segment)
- Measurand segments are added to the chip (washed across) → hybridization occurs if the measurand segments fit the segments on the chip.
- Measure the fluorescence of the individual squares on the chip to find out the amount of matching DNA.

Next generation sequencing techniques

- Pyrosequencing DNA
  - This technique does not stop the polymerisation but tries to record if anything happens with the DNA.
  - Relies on the light detection based on a chain reaction when pyrophosphate is released, using Sulfurylase and Luciferase.
  - Adding only one base at a time (can be achieved by microfluidics), it produces light if the base fits the end of the sequence.
  - Instead of terminating the chain with dideoxynucleotides, it detects the change of the chain length.
- SMRT method (Single Molecule Real Time)
  - Free bases are attached to fluorophore molecules.
  - When they bind to the DNA sequence, the marker detached from it. As soon as the base incorporates, there is fluorophore release near the base (??) → depending on the colour we know what type of nucleotide is incorporated.
  - External illumination stimulates the free marker → Colour detection → Base detection.
  - Detection in a well-like structure
    - Isolated from the rest of the solution → clearer signal
  - Difficulty: all nucleotides have to be separately fluorescently labelled.
- Ion Torrent
  - Ion selective FETs are used to detect small changes in pH.
  - Each nucleotide causes a different amount of pH change when it binds to the sequence, and CMOS measures pH change as nucleotide cycle through.
  - Nucleotides have to be introduced one-by-one.
- Nanopore methods
  - Single stranded DNA is passed through a nanopore (protein)
  - Nucleotides partially block the pore → restrict current flowing through it
  - Current can be measured → nucleotides can be differentiated

Summarize the basic properties of proteins! Explain proteomics and protein chips! List the applicable protein sequencing and identification methods!

Basic properties of proteins:

- Made up of amino acids (peptide bond)
- <50 amino acids → peptide; >50 → protein
- Properties are defined by their structure
    - Primary: the sequence of amino acids
    - Secondary: repeating local structures stabilized by H-bonds (α-helix, β-sheet, turns)
    - Tertiary (Fold): overall shape, relationship between secondary structural elements, stabilized by H-bond, disulphide-bond, post-translational modifications
    - Quaternary: multiple protein domains combine, usually they have specific functions
- Enzymes, transport proteins, storage proteins, motors (elements of cytoskeleton), structural proteins (e.g. keratin), antibodies, regulatory proteins (e.g. hormones), receptors

Proteomics:

- The study of all proteins, including their relative abundance, distribution, post-translational modifications, functions, and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.
- Abundance proteomics:
    - Relative abundance of specific proteins in a given tissue under different conditions of health and disease.
- Cell-mapping:
    - Intracellular signalling pathways and regulatory networks mediated through protein-protein interactions.
- Structural proteomics:
    - Study of active sites and functional domains of proteins to better understand processes such as enzyme catalysis, protein stability and interaction with multi- molecular complexes.
- Applications:
    - Mining: identification of proteins
    - Protein-expression profile: identification of proteins in a particular state of the organism
    - Protein-network mapping: protein interactions in living systems
    - Mapping of protein modifications: how and where proteins are modified

Protein microarrays:

- Useful for studying protein expression, interaction, function and post-translational modifications.
- High-throughput, high sensitivity, low sample volumes, and efficient sample-to-result time.
- Forward-phase microarrays:
    - Proteins and peptides are immobilized for capturing antibodies. Proteins and peptides are immobilized for capturing antibodies.
    - Antibodies, sugars or aptamers are immobilized and labelled proteins are captured.
    - Sandwich mode – a labelled secondary antibody is used for detection.
- Reverse phase microarrays:
    - Complex samples such as serum, plasma, or even tissues are immobilized in an array format and probed with antibodies to determine the differential amount of protein molecules in the screened samples.

- Applications:
    - Protein expression profiling, studying posttranslational modifications, protein-protein binding, drug interaction, protein folding, substrate specificity, enzymatic activity and interaction between proteins and nucleic acids
- Microarray analysis concept:
    - The sample is prepared and incubated with the microarray.
    - Previously, a corresponding binding partner for the molecule of interest was immobilized on the surface of the microarray.
    - Here, an interacting antibody and antigen are depicted.
    - Detection is performed by labelling a secondary antibody, which results in intensive signals if the molecule of interest is present.
- Array Formats
    - Proteins and peptides are immobilized as capturing agents for antibody detection, whereas an anti-immunoglobulin, a common labelled antibody, is used for detection.
    - Antibodies, sugars, or aptamers are immobilized, and labelled proteins are captured.
    - A labelled secondary antibody is used for detection.
    - Complex samples such as serum, plasma or tissues are immobilized in an array format and probed with antibodies to determine the differential amount of protein molecules in the screened samples.
- General Protein Microarray Types
    - Antibodies may be probed with cell lysate for determining protein expression levels as well as the specificity of the resultant interaction.
    - Can be used to study the biochemical properties and activities of target proteins.
    - Can be used for investigating posttranslational modifications and biomarker identification.
- Capture Molecules & applications:
    - Protein-protein: disease progression, signal-pathway studies
    - Enzyme – substrate: substrate binding analyses
    - Receptor – ligand: drug discovery
    - Antigen – antibody: biomarker identification in auto-immune diseases
    - Aptamers: protein-protein interaction analyses
- Array fabrication technologies:
    - Contact: pin printing, microstamps, flow printing
    - Lithography: photolithography, electron-beam, AFM lithography
    - Non-contact: thermal inject, piezo actuation, valve jet
    - Cell-Free: PISA, NAPPA, In situ puromycin capture
- Factors Affecting Performance
    - Appropriate surface for the immobilization of either protein or antibody samples.
    - Microarray patterning technique.
    - Protein conformational changes with expression, purification or immobilization may alter their function or render them inactive.
    - Charged surfaces, temperature, pH and solvents may denature some proteins, and therefore surfaces must be biocompatible to minimize denaturation.
    - Protein instability may lessen shelf-life.

Identification & Sequencing Methods

- Western blot
  - - separation of proteins according to their length and isoelectric point using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) followed by transfer to a membrane.
  - Detection of certain proteins with primary and secondary labelled antibodies
- ELISA
  - wet samples incubated with specific antibodies in a microtiter plate. Detection is in wells.
- Bead-based method
  - Similar to ELISA, but not only one angitgen is used → parallel testing of multiple samples
  - E.g. Luminex, a bead-based method that can analyze multiple samples in one experiment by utilizing different bead types.
- Mass spectrometry
  - Sample enters the chamber (maybe already digested)
  - Charge is added to the sample
    - Electron bombardment or vaporization
  - Accelerating the particles towards a magnet using an electric field
  - Magnetic field exerts force on the charged particles → particle beam bends
  - Bending is influenced by the particle's mass-to-charge ratio
  - Particles hit a detector → based on the bending they can be differentiated
  - By knowing the composition of the sample, we get a list of possible amino acid sequences
- Matrix-assisted laser desorption/ionization (MALDI)
  - In mass spectrometry MALDI is an ionization technique that uses a laser energy absorbing matrix to create ions from large molecules with minimal fragmentation
  - Sample is mixed with a suitable matrix material and applied to a metal plate
  - Pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material
  - The analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases
  - Accelerated into mass spectrometer
- Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE) S
  - Separating proteins based on mass and isoelectric point
  - SDS is used to unfold the proteins by breaking the H-bonds between segments
  - Proteins are transferred to the gel where an electric field is applied
  - Negatively charged proteins migrate towards the anode
  - Small proteins travel faster
  - Then staining or western blotting reveals the outcome
  - The distance travelled by different proteins is compared to added samples with known molecular weights

List at least 4 commercially available BioMEMS devices, operational principles, and evaluate these devices critically! What problems do they provide the solution for?

Lung on a chip

- Microdevice that has the potential to replace animals for the testing of drugs and toxins.
- Can bring new therapies to patients faster and at lower cost.
- Mimics the mechanical and chemical functions of a living, breathing lung.
- Lined with human cells. (Lung & capillary cells.)
- Contains tiny hollow channels created using microchip fabrication techniques.
- Cyclic suction + side channels make the entire flexible sheath and cells stretch and relax rhythmically, jut like lung cells during breathing.
- Air flows over the top of the human lung cells, and a liquid medium containing human white blood cells flows below the capillary cell layer.

Translational Organ-on-Chip Platform

- Organ-on-Chip devices mimic the native environment of the cells including 3D topology or physical stretch and strain.
- Aim: standardizing the way Organ-on-Chips are being controlled and analyzed without compromise, user or developer flexibility.
- TOP makes life easier with active switchable integrated microfluidics, which can be used to control the fluidics from, ,to and within the chips.
- Biological protocols can be run automatically with the click of a button.

Multi-Electrode Array Chip Technology

- Capable of simultaneous on chip measurements.
- High density patches of micro-sized electrodes.
- Offers single cell resolution and unprecedented signal quality.
- The chip can be packed in a microfluidic well plate for cell culturing.
- On the chip, the cells connect to the electrodes, that read out the intracellular and extracellular signals.
- Can also be used for precise cell programming. By applying voltage to the cell, the membrane is electroporated and molecules and solutions can enter. → Cells can be differentiated in a controlled arrangement. → We can build tissue models on chips, that mimic the properties of the actual organ.

i-Stat handheld blood analyser

- Patient-side, lab accurate blood tests are available in minutes.
- Eliminates re-sticks due to delays with multi-use analysers.
- Minimising the risk of sample clotting before or during testing.
- Consolidating analytes on one cartridge.