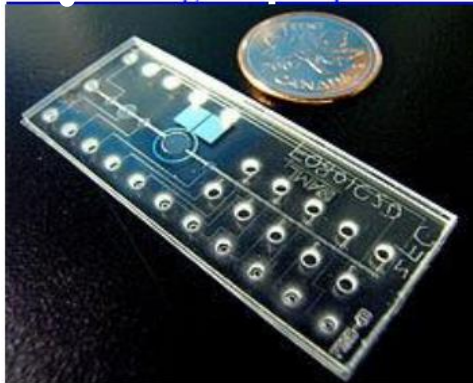
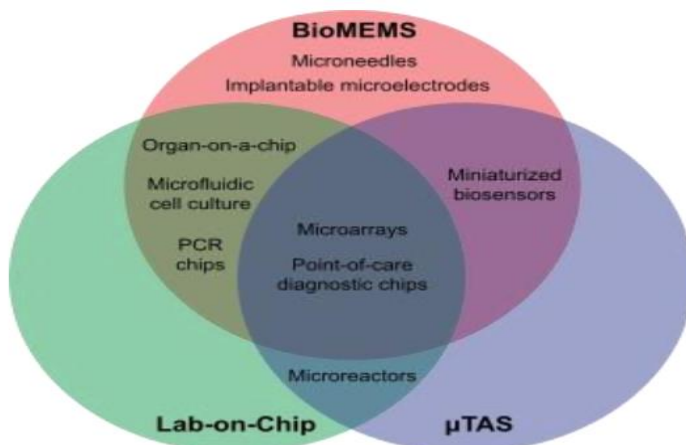


CONCLUSION: PRACTICAL APPLICATIONS



An example of a bio-MEMS device is this automated [FISH](#) microchip, which integrates a reagent multiplexer, a cell chamber with a thin-film heater layer, and a peristaltic pump.

Bio-MEMS is an abbreviation for [biomedical](#) (or [biological](#)) [microelectromechanical systems](#). Bio-MEMS have considerable overlap, and is sometimes considered synonymous, with [lab-on-a-chip \(LOC\)](#) and [micro total analysis systems \(\$\mu\$ TAS\)](#). Bio-MEMS is typically more focused on mechanical parts and [microfabrication](#) technologies made suitable for biological applications. On the other hand, [lab-on-a-chip](#) is concerned with [miniaturization](#) and integration of laboratory processes and experiments into single (often [microfluidic](#)) chips. In this definition, lab-on-a-chip devices do not strictly have biological applications, although most do or are amenable to be adapted for biological purposes. Similarly, micro total analysis systems may not have biological applications in mind, and are usually dedicated to [chemical analysis](#). A broad definition for bio-MEMS can be used to refer to the science and technology of operating at the microscale for biological and biomedical applications, which may or may not include any electronic or mechanical functions. The interdisciplinary nature of bio-MEMS combines [material sciences](#), [clinical sciences](#), [medicine](#), [surgery](#), [electrical engineering](#), [mechanical engineering](#), [optical engineering](#), [chemical engineering](#), and [biomedical engineering](#). Some of its major applications include [genomics](#), [proteomics](#), [molecular diagnostics](#), [point-of-care diagnostics](#), [tissue engineering](#), [single cell analysis](#) and implantable microdevices.



A [Venn diagram](#) outlining and contrasting some aspects of the fields of bio-MEMS, lab-on-a-chip, μ TAS.

Approaches

Materials

Silicon and glass

Conventional micromachining techniques such as [wet etching](#), dry etching, deep reactive ion etching, [sputtering](#), [anodic bonding](#), and [fusion bonding](#) have been used in bio-MEMS to make [flow channels](#), [flow sensors](#), chemical detectors, separation capillaries, mixers, [filters](#), [pumps](#) and valves. However, there are some drawbacks to using silicon-based devices in biomedical applications such as their high cost and [bioincompatibility](#). Due to being single-use only, larger than their [MEMS](#) counterparts, and the requirement of [clean room](#) facilities, high material and processing costs make [silicon](#)-based bio-MEMS less economically attractive. “[In vivo](#)”, silicon-based bio-MEMS can be readily functionalized to minimize [protein adsorption](#), but the brittleness of silicon remains a major issue.

Plastics and polymers

Using [plastics](#) and [polymers](#) in bio-MEMS is attractive because they can be easily fabricated, compatible with micromachining and [rapid prototyping](#) methods, as well as have low cost. Many polymers are also [optically transparent](#) and can be integrated into systems that use optical

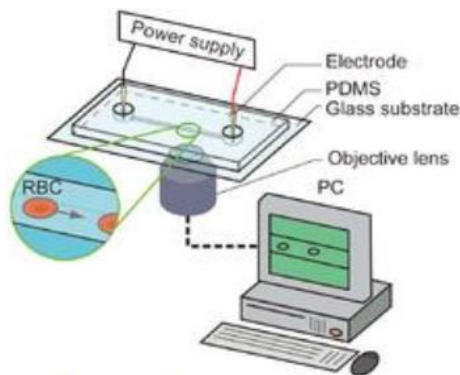
detection techniques such as [fluorescence](#), [UV/Vis absorbance](#), or [Raman method](#). Moreover, many polymers are [biologically compatible](#), chemically inert to [solvents](#), and [electrically insulating](#) for applications where strong [electrical fields](#) are necessary such as [electrophoretic separation](#). [Surface chemistry](#) of [polymers](#) can also be modified for specific applications. Specifically, the surface of [PDMSs](#) can be [ion-irradiated](#) with elements such as [magnesium](#), [tantalum](#), and [iron](#) to decrease surface [hydrophobicity](#), allowing for better cell adhesion in “[in vivo](#)” applications. The most common polymers used in bio-MEMS include [PMMA](#), [PDMS](#), [OSTEmer](#) and [SU-8](#).

Paper

Paper microfluidics (sometimes called lab on paper) is the use of paper substrates in [microfabrication](#) to manipulate fluid flow for different applications. Paper microfluidics have been applied in paper electrophoresis and [immunoassays](#), the most notable being the commercialized pregnancy test, ClearBlue. Advantages of using paper for [microfluidics](#) and [electrophoresis](#) in bio-MEMS include its low cost, [biodegradability](#), and natural [wicking](#) action. A severe disadvantage of paper-based [microfluidics](#) is the dependency of the rate of wicking on environmental conditions such as temperature and relative humidity. Paper-based

analytical devices are particularly attractive for point-of-care diagnostics in developing countries for both the low material cost and emphasis on colorimetric assays which allow medical professionals to easily interpret the results by eye. Compared to traditional microfluidic channels, paper microchannels are accessible for sample introduction (especially [forensic](#)-style samples such as body fluids and soil), as well as its natural filtering properties that exclude cell debris, dirt, and other impurities in samples. Paper-based replicas have demonstrated the same effectiveness in performing common [microfluidic](#) operations such as [hydrodynamic focusing](#), size-based molecular extraction, [micro-mixing](#), and dilution; the common 96- and 384-well [microplates](#) for [automated liquid handling and analysis](#) have been reproduced through photolithography on paper to achieve a slimmer profile and lower material cost while maintaining compatibility with conventional [microplate](#) readers. Techniques for [micropatterning](#) paper include [photolithography](#), [laser cutting](#), ink jet printing, [plasma treatment](#), and wax patterning.

Electrokinetics

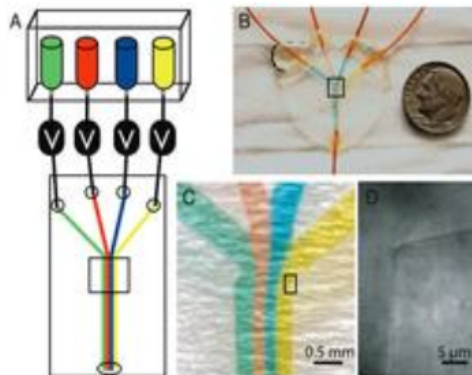


An [electrophoresis](#) experiment example: Two conical [electrodes](#) are set at both the inlet and outlet of a [microchannel](#) and cells are moved along the microchannel by an applied DC [electric field](#).

Electrokinetics have been exploited in bio-MEMS for separating mixtures of molecules and [cells](#) using electrical fields. In [electrophoresis](#), a charged species in a liquid moves under the influence of an applied [electric field](#). [Electrophoresis](#) has been used to fractionate small [ions](#), charged organic molecules, [proteins](#), and [DNA](#). Electrophoresis and microfluidics are highly synergistic because it is possible to use higher voltages in microchannels due to faster [heat removal](#). [Isoelectric focusing](#) is the separation of proteins, [organelles](#), and cells with different [isoelectric points](#). Isoelectric focusing requires a [pH](#) gradient (usually generated with [electrodes](#)) perpendicular to the flow direction. Sorting and focusing of the species of interest is achieved because an electrophoretic force causes perpendicular migration until it flows along its respective isoelectric points. [Dielectrophoresis](#) is the motion of uncharged particles due to induced polarization from nonuniform electric fields. Dielectrophoresis can be used in bio-MEMS for dielectrophoresis traps, concentrating specific particles at specific points on surfaces, and diverting particles from one flow stream to another for dynamic concentration. -

Microfluidics

Microfluidics refers to systems that manipulate small (μL , nL , pL , fL) amounts of fluids on microfabricated substrates. Microfluidic approaches to bio-MEMS confer several advantages:



When multiple solutions are added into the same [microchannel](#), they flow in separate flow lanes (no mixing) due to [laminar flow](#) characteristics.

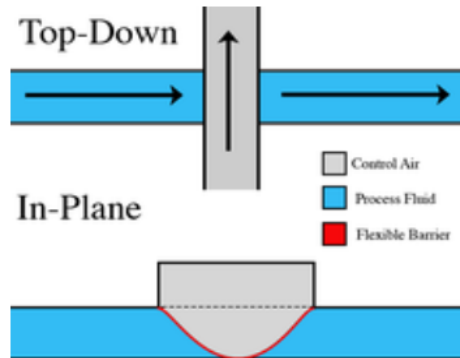
- Flow in microchannels is laminar, which allows selective treatment of cells in microchannels, [mathematical modelling](#) of flow patterns and [concentrations](#), as well as quantitative predictions of the biological environment of cells and biochemical reactions
- Microfluidic features can be fabricated on the cellular scale or smaller, which enables investigation of (sub)cellular phenomena, seeding and sorting of single cells, and recapitulation of physiological parameters
- Integration of [microelectronics](#), [micromechanics](#), and microoptics onto the same platform allows [automated device control](#), which reduces human error and operation costs
- Microfluidic technology is relatively economical due to batch fabrication and high-throughput (parallelization and redundancy). This allows the production of disposable or single-use chips for improved ease of use and reduced probability of biological [cross contamination](#), as well as [rapid prototyping](#)
- Microfluidic devices consume much smaller amounts of [reagents](#), can be made to require only a small amount of [analytes](#) for chemical detection, require less time for processes and reactions to complete, and produces less waste than conventional macrofluidic devices and experiments
- Appropriate packaging of microfluidic devices can make them suitable for wearable applications, [implants](#), and portable applications in [developing countries](#)

An interesting approach combining electrokinetic phenomena and microfluidics is [digital microfluidics](#). In digital microfluidics, a substrate surface is [micropatterned](#) with [electrodes](#) and selectively activated. Manipulation of small fluid droplets occurs via [electrowetting](#), which is the phenomenon where an electric field changes the [wettability](#) of an electrolyte droplet on a surface.

BioMEMs Flow Control

Lithographic methods for microfluidic device manufacturing are ineffective in forming the screw-type mechanisms used in macroscale valves. Therefore, microfluidic devices require alternative flow control techniques, a number of which are currently popular:

Quake Valves



Schematic of a quake valve, with process fluid channel perpendicular and out of plane with the control fluid channel.

One inexpensive method of producing valves with fast actuation times and variable flow restriction is multilayer soft lithography (MSL). Valves produced through this fabrication technique are called Quake valves, because they were first created in the lab of [Stephen Quake](#) at [Stanford University](#). The basic scheme involves two perpendicular flow conduits separated by an impermeable elastomeric membrane at their intersection. Controlled air flow passes through one conduit while the process fluid passes through the other. A pressure gradient between the two conduits, which is tuned by changing the control air flow rate, causes the membrane to deform and obstruct flow in the process channel. In MSL, the channels for both the process fluid and the control fluid are cast out of an [elastomeric](#) mold, making it an entirely additive manufacturing process.

Ice Valves

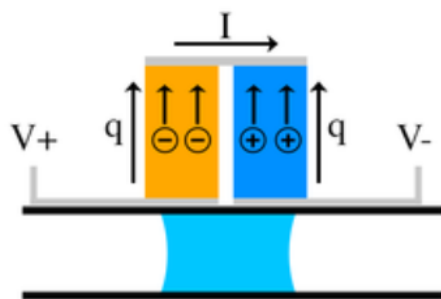


Diagram of an ice valve with Peltier cooling element.

Ice valves operate by transporting heat away from a single portion of a flow channel, causing the fluid to solidify and stop flow through that region. [Thermoelectric](#) (TE) units are used to transport heat away from the plug. Because of the limited temperature difference that TE units can provide, multiple are often chained in series to produce subzero temperatures at the

substrate-fluid interface, allowing for more rapid cooling. Current state of the art ice valve technology features short closing times (0.37 s at 10 $\mu\text{L}/\text{min}$) and also operates at high flow rates (1150 $\mu\text{L}/\text{min}$). Ice valves were first introduced in 1995 where pressurized liquid [carbon dioxide](#) was used as the cooling agent.

Prefabricated Valves

Prefabricated mechanical screw valves and solenoid valves require no advanced microfabrication processes and are easy to implement in soft substrate materials like [PDMS](#). Screw valves, unlike Quake and ice valves, maintain their level of flow restriction without power input, and are thus ideal for situations where the valve position may remain mostly constant and actuation by a human operator is acceptable. Electromagnetic solenoid valves have similar actuation times compared to Quake valves, but have larger footprints and are not integrated into the device substrate. This is an issue when device dimensions are an issue, such as in implantable devices.

Micro-scale Mixing

Despite the fact that diffusion times are significantly higher in microfluidic systems due to small length scales, there are still challenges to removing concentration gradients at the time scales required for microfluidic technologies.

Sonication Mixing Elements



A passive flow mixing element. Laminar flow with axial concentration gradients flows in, and laminar flow with diminished concentration gradients flows out.

[Sonication](#) is often employed to provide local mixing of streams through the generation of ultra-high energy acoustics. Microfluidic chips utilizing sonication mixing can have both [integrated](#) and externally located ultrasonic transducers. Sonication is also used widely for cell lysis and homogenization in both macro and microfluidic systems. The primary mechanism of [cell lysis](#) by sonication is intense local heating and [shear forces](#).

Passive Mixing Elements

In a passive mixing element, mixing is achieved by temporal and spatial redistribution of incoming [laminar flow](#) through the use of parallel conduits of variable path length and or diameter. The net result of having a variety of parallel flow channels of varying length is that material initially at the edge of the laminar flow profile can be repeatedly redistributed to the opposite edge, thus drastically shortening the characteristic diffusion length scale.

Bio-MEMS as Miniaturized Biosensors

Main article: [Biosensor](#)

Biosensors are devices that consist of a biological recognition system, called the bioreceptor, and a [transducer](#). The interaction of the [analyte](#) with the bioreceptor causes an effect that the transducer can convert into a measurement, such as an [electrical signal](#). The most common bioreceptors used in biosensing are based on [antibody–antigen](#) interactions, [nucleic acid](#) interactions, [enzymatic](#) interactions, cellular interactions, and interactions using [biomimetic materials](#). Common transducer techniques include mechanical detection, electrical detection, and optical detection.

Micromechanical sensors

Mechanical detection in bio-MEMS is achieved through micro- and nano-scale [cantilevers](#) for [stress](#) sensing and mass sensing, or micro- and nano-scale plates or membranes. In stress sensing, the biochemical reaction is performed selectively on one side of the cantilever to cause a change in [surface free energy](#). This results in bending of the cantilever that is measurable either optically ([laser](#) reflection into a quadposition detector) or electrically ([piezo-resistor](#) at the fixed edge of the cantilever) due to a change in surface stress. In mass sensing, the cantilever vibrates at its [resonant frequency](#) as measured electrically or optically. When a biochemical reaction takes place and is captured on the cantilever, the mass of the cantilever changes, as does the resonant frequency. Analysis of this data can be slightly less straightforward, however, as adsorption of sample to the cantilever has also been found to change the Young's modulus of the cantilever. Changing cantilever stiffness will also change its resonant frequency, and thus the noise in the oscillation signal must be analyzed to determine whether the resonant frequency is also a function of changing elasticity. One common use for this technique is in detecting nucleotide mismatches in DNA because the variation in mass caused by the presence of an incorrect base is enough to change the resonant frequency of the cantilever and register a signal. Mass sensing is not as effective in fluids because the minimum detectable mass is much higher in [damped mediums](#). Suspended microchannel resistors are a special type of cantilever design that are able to work around this limitation using microfluidic channels inside the cantilever. These channels can move "in situ" samples around on the cantilever, without submerging the cantilever, minimally impacting its oscillation. This technology is in its infancy, however, and it is still not able to be used beyond a few, limited applications. The advantage of using cantilever sensors is that there is no need for an optically detectable label on the [analyte](#) or bioreceptors.

Electrical and electrochemical sensors

Electrical and [electrochemical](#) detection are easily adapted for portability and [miniaturization](#), especially in comparison to optical detection. In [amperometric](#) biosensors, an [enzyme](#)-catalyzed [redox](#) reaction causes a redox electron [current](#) that is measured by a working electrode. Amperometric biosensors have been used in bio-MEMS for detection of [glucose](#), [galactose](#), [lactose](#), [urea](#), and [cholesterol](#), as well as for applications in [gas](#) detection and [DNA hybridization](#). In [potentiometric](#) biosensors, measurements of electric potential at one

electrode are made in reference to another electrode. Examples of potentiometric biosensors include [ion-sensitive field effect transistors \(ISFET\)](#), [Chemical field-effect transistors \(chem-FET\)](#), and [light-addressable potentiometric sensors \(LAPS\)](#). In [conductometric biosensors](#), changes in [electrical impedance](#) between two electrodes are measured as a result of a biomolecular reaction. Conductive measurements are simple and easy to use because there is no need for a specific reference electrode, and have been used to detect biochemicals, [toxins](#), [nucleic acids](#), and [bacterial cells](#).

Optical sensors

A challenge in optical detection is the need for integrating detectors and [photodiodes](#) in a miniaturized portable format on the bio-MEMS. Optical detection includes [fluorescence](#)-based techniques, [chemiluminescence](#)-based techniques, and [surface plasmon resonance \(SPR\)](#). Fluorescence-based optical techniques use markers that emit light at specific [wavelengths](#) and the presence or enhancement/reduction (e.g. [fluorescence resonance energy transfer](#)) in optical signal indicates a reaction has occurred. Fluorescence-based detection has been used in [microarrays](#) and [PCR](#) on a chip devices. Chemiluminescence is [light](#) generation by energy release from a chemical reaction. [Bioluminescence](#) and [electrochemiluminescence](#) are subtypes of chemiluminescence. Surface plasmon resonance sensors can be thin-film [refractometers](#) or gratings that measure the resonance behaviour of [surface plasmon](#) on metal or dielectric surfaces. The resonance changes when biomolecules are captured or adsorbed on the sensor surface and depends on the concentration of the analyte as well as its properties. Surface plasmon resonance has been used in [food quality and safety analysis](#), [medical diagnostics](#), and [environmental monitoring](#).

Bio-MEMS for diagnostics

Genomic and proteomic microarrays



Affymetrix GeneChip® is an example of a genomic microarray.

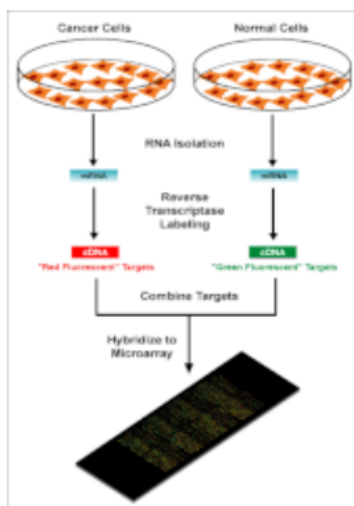
The goals of [genomic](#) and [proteomic](#) microarrays are to make high-throughput [genome](#) analysis faster and cheaper, as well as identify activated [genes](#) and their sequences. There are many different types of biological entities used in microarrays, but in general the microarray consists of an ordered collection of microspots each containing a single defined molecular species that interacts with the analyte for simultaneous testing of thousands of parameters in a single experiment. Some applications of genomic and proteomic microarrays are [neonatal screening](#), identifying disease risk, and predicting therapy efficacy for [personalized medicine](#).

Oligonucleotide chips

Oligonucleotide chips are microarrays of [oligonucleotides](#). They can be used for detection of mutations and expression monitoring, and gene discovery and mapping. The main methods for creating an oligonucleotide microarray are by gel pads ([Motorola](#)), [microelectrodes](#) (Nanogen), [photolithography](#) ([Affymetrix](#)), and [inkjet technology](#) ([Agilent](#)).

- Using gel pads, prefabricated oligonucleotides are attached to patches of activated [polyacrylamide](#)
- Using [microelectrodes](#), negatively charged DNA and molecular probes can be concentrated on energized electrodes for interaction
- Using photolithography, a light exposure pattern is created on the substrate using a [photomask](#) or virtual photomask projected from a [digital micromirror device](#). The light removes photolabile protecting groups from the selected exposure areas. Following de-protection, [nucleotides](#) with a photolabile protecting group are exposed to the entire surface and the chemical coupling process only occurs where light was exposed in the previous step. This process can be repeated to synthesize oligonucleotides of relatively short lengths on the surface, nucleotide by nucleotide.
- Using inkjet technology, nucleotides are printed onto a surface drop by drop to form oligonucleotides

cDNA microarray



Differential comparison in cDNA microarray

[cDNA](#) microarrays are often used for large-scale screening and expression studies. In cDNA microarrays, mRNA from cells are collected and converted into cDNA by reverse transcription. Subsequently, cDNA molecules (each corresponding to one gene) are immobilized as ~100 µm diameter spots on a membrane, glass, or [silicon](#) chip by metallic pins. For detection, fluorescently-labelled single strand cDNA from cells hybridize to the molecules on the microarray and a differential comparison between a treated sample (labelled red, for example) and an untreated sample (labelled in another color such as green) is used for analysis. Red dots mean that the corresponding gene was expressed at a higher level in the treated sample. Conversely, green dots mean that the corresponding gene was expressed at a higher level in the untreated sample. Yellow dots, as a result of the overlap between red and green dots, mean that the corresponding gene was expressed at relatively the same level in both samples, whereas dark spots indicate no or negligible expression in either sample.

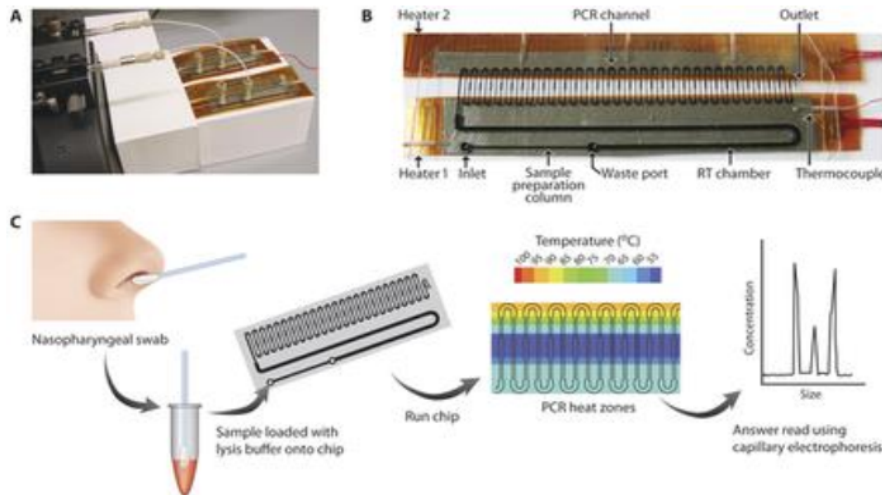
Peptide and protein microarrays

The motivation for using [peptide](#) and [protein microarrays](#) is firstly because [mRNA](#) transcripts often correlate poorly with the actual amount of protein synthesized. Secondly, [DNA microarrays](#) cannot identify post-translational modification of proteins, which directly influences protein function. Thirdly, some bodily fluids such as urine lack [mRNA](#). A protein microarray consists of a protein library immobilized on a substrate chip, usually glass, silicon, [polystyrene](#), [PVDF](#), or [nitrocellulose](#). In general, there are three types of protein microarrays: functional, analytical or capture, and reverse-phase protein arrays.

- Functional protein arrays display folded and active proteins and are used for screening molecular interactions, studying protein pathways, identifying targets for [post-translational modification](#), and analyzing [enzymatic activities](#).
- Analytical or capture protein arrays display antigens and [antibodies](#) to profile protein or antibody expression in serum. These arrays can be used for [biomarker discovery](#), monitoring of protein quantities, monitoring activity states in [signalling pathways](#), and profiling antibody repertoires in diseases.
- Reverse-phase protein arrays test replicates of cell lysates and [serum](#) samples with different antibodies to study the changes in expression of specific proteins and protein modifications during disease progression, as well as [biomarker discovery](#).

Protein microarrays have stringent production, storage, and experimental conditions due to the low stability and necessity of considering the native folding on the immobilized proteins. Peptides, on the other hand, are more chemically resistant and can retain partial aspects of protein function. As such, peptide microarrays have been used to complement protein microarrays in proteomics research and diagnostics. Protein microarrays usually use [Escherichia coli](#) to produce proteins of interest; whereas peptide microarrays use the SPOT technique (stepwise synthesis of peptides on cellulose) or photolithography to make peptides.

PCR chips



Continuous flow-based [PCR microfluidic](#) system with thin film heaters, [syringe pump](#), and continuous flow [PCR channel](#). The application of this example bio-MEMS is for amplification of [influenza A](#) RNA in respiratory specimens

The [polymerase chain reaction \(PCR\)](#) is a fundamental [molecular biology](#) technique that enables the selective [amplification](#) of [DNA](#) sequences, which is useful for expanded use of rare samples e.g.: stem cells, biopsies, circulating tumor cells. The reaction involves [thermal cycling](#) of the DNA sequence and [DNA polymerase](#) through three different temperatures. Heating up and cooling down in conventional PCR devices are time-consuming and typical PCR reactions can take hours to complete. Other drawbacks of conventional PCR is the high consumption of expensive reagents, preference for amplifying short fragments, and the production of short chimeric molecules. PCR chips serve to miniaturize the reaction environment to achieve rapid [heat transfer](#) and fast mixing due to the larger surface-to-volume ratio and short [diffusion](#) distances. The advantages of PCR chips include shorter thermal-cycling time, more uniform temperature which enhances yield, and portability for point-of-care applications. Two challenges in microfluidic PCR chips are PCR inhibition and contamination due to the large surface-to-volume ratio increasing surface-reagent interactions. For example, silicon substrates have good [thermal conductivity](#) for rapid heating and cooling, but can poison the polymerase reaction. Silicon substrates are also opaque, prohibiting optical detection for qPCR, and electrically conductive, preventing electrophoretic transport through the channels. Meanwhile, glass is an ideal material for electrophoresis but also inhibits the reaction. Polymers, particularly [PDMS](#), are optically transparent, not inhibitory, and can be used to coat an electrophoretic glass channel. Various other surface treatments also exist, including polyethylene glycol, bovine serum albumin, and silicon dioxide. There are stationary (chamber-based), dynamic (continuous flow-based), and microdroplet ([digital PCR](#)) chip architectures.

- Chamber-based architecture is the result of shrinking down of conventional PCR reactors, which is difficult to scale up. A four-layer glass-[PDMS](#) device has been developed using this architecture integrating microvalves, microheaters, temperature sensors, 380-nL reaction chambers, and [capillary electrophoresis](#) channels for [reverse transcription polymerase chain reaction \(RT-PCR\)](#) that has [attomolar](#) detection sensitivity.

- Continuous flow-based architecture moves the sample through different temperature zones to achieve [thermal cycling](#). This approach uses less energy and has high throughput, but has large reagent consumption and gas bubbles can form inside the flow channels.
- [Digital PCR](#) eliminates sample/reagent surface [adsorption](#) and contamination by carrying out PCR in microdroplets or microchambers. PCR in droplets also prevents recombination of homologous gene fragments so synthesis of short chimeric products is eliminated.

Point-of-care-diagnostic devices



A midwife draws blood to measure patients' [CD4](#) count within 20 minutes using the Pima point-of-care CD4 analyzer, in Uganda.

The ability to perform medical diagnosis at the bedside or at the point-of-care is important in health care, especially in developing countries where access to centralized hospitals is limited and prohibitively expensive. To this end, point-of-care diagnostic bio-MEMS have been developed to take saliva, blood, or urine samples and in an integrated approach perform sample preconditioning, sample fractionation, signal amplification, analyte detection, data analysis, and result display. In particular, blood is a very common biological sample because it cycles through the body every few minutes and its contents can indicate many aspects of health.

Sample conditioning

In blood analysis, [white blood cells](#), [platelets](#), [bacteria](#), and [plasma](#) must be separated. Sieves, weirs, inertial confinement, and flow diversion devices are some approaches used in preparing blood plasma for cell-free analysis. Sieves can be microfabricated with high-aspect-ratio columns or posts, but are only suitable for low loading to avoid clogging with cells. Weirs are shallow mesa-like sections used to restrict flow to narrow slots between layers without posts. One advantage of using weirs is that the absence of posts allows more effective recycling of retentate for flow across the filter to wash off clogged cells. Magnetic beads are used to aid in analyte separation. These microscopic beads are functionalized with target molecules and moved through microfluidic channels using a varying magnetic field. This serves as a quick method of harvesting targets for analysis. After this process is complete, a strong, stationary magnetic field is applied to immobilize the target-bound beads and wash away unbound beads. The H-filter is a microfluidic device with two inlets and two outlets that takes advantage of [laminar flow](#) and diffusion to separate components that diffuse across the interface between two inlet

streams. By controlling the flow rate, diffusion distance, and residence time of the fluid in the filter, cells are excluded from the filtrate by virtue of their slower diffusion rate. The H-filter does not clog and can run indefinitely, but analytes are diluted by a factor of two. For cell analysis, cells can be studied intact or after [lysis](#). A lytic buffer stream can be introduced alongside a stream containing cells and by diffusion induces lysis prior to further analysis. Cell analysis is typically done by [flow cytometry](#) and can be implemented into [microfluidics](#) with lower fluid velocities and lower throughput than their conventional macroscopic counterparts.

Sample fractionation

Microfluidic sample separation can be achieved by [capillary electrophoresis](#) or continuous-flow separation. In capillary electrophoresis, a long thin tube separates analytes by voltage as they migrate by [electro-osmotic](#) flow. For continuous-flow separation, the general idea is to apply a field at an angle to the flow direction to deflect the sample flow path toward different channels. Examples of continuous-flow separation techniques include continuous-flow electrophoresis, [isoelectric focusing](#), continuous-flow magnetic separations, and [molecular sieving](#).

Outstanding Challenges

- Most diagnostic devices on the market can only test for one disease. Moreover most devices are binary output (yes/no) without nuanced information on the patient's condition. Thus, in addition to developing tests for more diseases, scientists are currently working to expand the complexity of these devices, in order to increase their utility.
- It is difficult to manufacture MEMS diagnostic devices outside of the laboratory setting. Much of the research on these devices takes place in climate controlled laboratories, where the devices can be tested shortly after they are produced. However, as many of these devices are used to screen for tropical diseases, they must be robust enough to survive in hot, humid conditions. They must also be stored for long periods from the time of production to the time of use.
- Funding is scarce for tropical disease research. In addition, there are many regulatory hurdles that must be cleared before a medical device is approved, which can cost tens of millions of dollars. Thus, companies focusing on tropical diseases must often combine their research objectives for tropical disease with research on other, more well-funded areas of medical research.

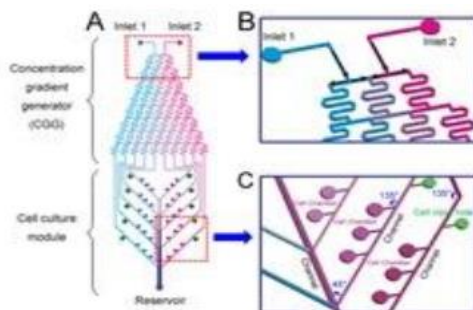
Bio-MEMS in tissue engineering

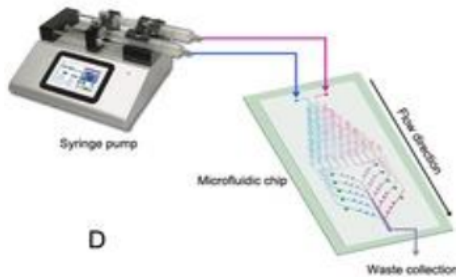
Cell culture

Conventional [cell culture](#) technology is unable to efficiently allow combinatorial testing of drug candidates, [growth factors](#), [neuropeptides](#), genes, and [retroviruses](#) in cell culture medium. Due to the need for cells to be fed periodically with fresh medium and passaged, even testing a few conditions requires a large number of cells and supplies, expensive and bulky [incubators](#), large fluid volumes (~0.1 – 2 mL per sample), and tedious human labour. The requirement of human labour also limits the number and length between time points for experiments. Microfluidic cell cultures are potentially a vast improvement because they can be automated, as well as yield

lower overall cost, higher throughput, and more quantitative descriptions of single-cell behaviour variability. By including [gas exchange](#) and temperature control systems on chip, microfluidic cell culturing can eliminate the need for incubators and [tissue culture hoods](#). However, this type of continuous microfluidic cell culture operation presents its own unique challenges as well. Flow control is important when seeding cells into microchannels because flow needs to be stopped after the initial injection of cell suspension for cells to attach or become trapped in microwells, dielectrophoretic traps, micromagnetic traps, or hydrodynamic traps. Subsequently, flow needs to be resumed in a way that does not produce large forces that [shear](#) the cells off the substrate. Dispensing fluids by [manual](#) or [robotic pipetting](#) can be replaced with [micropumps](#) and microvalves, where fluid metering is straightforward to determine as opposed to continuous flow systems by micromixers. A fully automated microfluidic [cell culture](#) system has been developed to study osteogenic differentiation of human [embryonic stem cells](#). A handheld microfluidic cell culture incubator capable of heating and pumping cell culture solutions has also been developed. Due to the volume reduction in [microfluidic](#) cultures, the collected concentrations are higher for better [signal-to-noise ratio](#) measurements, but collection and detection is correspondingly more difficult. ["In situ" microscopy assays](#) with microfluidic cell cultures may help in this regard, but have inherently lower throughput due to the microscope probe having only a small field of view. The Berkeley Lights Beacon platform has resolved the issue of collection and detection by performing [microfluidic](#) culture on an array of [photoconductors](#) which can be [optoelectrically](#) activated to manipulate cells across the chip. This platform has been adopted by [Amgen](#) and [Novartis](#) for cell line development in the biopharmaceutical industry. [Micropatterned](#) co-cultures have also contributed to bio-MEMS for [tissue engineering](#) to recapitulate *in vivo* conditions and 3D natural structure. Specifically, [hepatocytes](#) have been patterned to co-culture at specific cell densities with [fibroblasts](#) to maintain [liver-specific](#) functions such as [albumin](#) secretion, [urea](#) synthesis, and [p450](#) detoxification. Similarly, integrating microfluidics with [micropatterned](#) co-cultures has enabled modelling of [organs](#) where multiple vascularized tissues interface, such as the [blood-brain barrier](#) and the lungs. Organ-level lung functions have been reconstituted on [lung-on-a-chip](#) devices where a porous membrane and the seeded [epithelial cell](#) layer are cyclically stretched by applied vacuum on adjacent microchannels to mimic [inhalation](#).

Stem-cell engineering





An integrated microfluidic device with a concentration gradient generator and individual cell chambers for studying dose-dependent effects of [differentiation](#) inducing factors.

The goal of [stem cell](#) engineering is to be able to control the differentiation and [self-renewal](#) of pluripotency stem cells for [cell therapy](#). Differentiation in stem cells is dependent on many factors, including soluble and biochemical factors, fluid [shear stress](#), cell-[ECM](#) interactions, cell-cell interactions, as well as [embryoid body](#) formation and organization. Bio-MEMS have been used to research how to optimize the culture and growth conditions of stem cells by controlling these factors. Assaying stem cells and their differentiated progeny is done with microarrays for studying how [transcription factors](#) and [miRNAs](#) determine cell fate, how [epigenetic](#) modifications between stem cells and their daughter cells affect [phenotypes](#), as well as measuring and sorting stem cells by their protein expression.

Biochemical factors

Microfluidics can leverage its microscopic volume and laminar flow characteristics for spatiotemporal control of biochemical factors delivered to stem cells. Microfluidic gradient generators have been used to study [dose-response](#) relationships. [Oxygen](#) is an important biochemical factor to consider in differentiation via [hypoxia-induced transcription factors \(HIFs\)](#) and related signaling pathways, most notably in the development of blood, [vasculature](#), [placental](#), and bone tissues. Conventional methods of studying oxygen effects relied on setting the entire incubator at a particular oxygen concentration, which limited analysis to pair-wise comparisons between normoxic and hypoxic conditions instead of the desired concentration-dependent characterization. Developed solutions include the use of continuous axial oxygen gradients and arrays of microfluidic cell culture chambers separated by thin [PDMS](#) membranes to gas-filled [microchannels](#).

Fluid shear stress

Fluid [shear stress](#) is relevant in the stem cell differentiation of cardiovascular lineages as well as late [embryogenesis](#) and [organogenesis](#) such as left-right asymmetry during development. Macro-scale studies do not allow quantitative analysis of shear stress to differentiation because they are performed using [parallel-plate flow chambers](#) or rotating cone apparatuses in on-off scenarios only. [Poiseuille flow](#) in microfluidics allows shear stresses to be varied systematically using channel geometry and flow rate via [micropumps](#), as demonstrated by using arrays of perfusion chambers for [mesenchymal stem cells](#) and [fibroblast cell adhesion](#) studies.

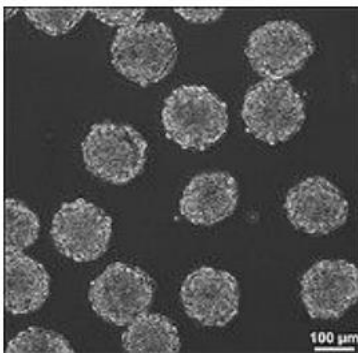
Cell-ECM interactions

Cell-ECM interactions induce changes in differentiation and self-renewal by the stiffness of the substrate via [mechanotransduction](#), and different [integrins](#) interacting with ECM molecules. [Micropatterning](#) of ECM proteins by [micro-contact printing \(μCP\)](#), [inkjet printing](#), and mask spraying have been used in [stem cell-ECM](#) interaction studies. It has been found by using micro-contact printing to control [cell attachment](#) area that that switch in osteogenic / adipogenic lineage in human [mesenchymal stem cells](#) can be cell shape dependent. [Microfabrication](#) of microposts and measurement of their [deflection](#) can determine traction forces exerted on cells. [Photolithography](#) can also be used to cross-link cell-seeded photo-polymerizable ECM for three-dimensional studies. Using [ECM microarrays](#) to optimize combinatorial effects of [collagen](#), [laminin](#), and [fibronectin](#) on stem cells is more advantageous than conventional [well plates](#) due to its [higher throughput](#) and lower requirement of expensive reagents.

Cell-cell interactions

[Cell fate](#) is regulated by both interactions between [stem cells](#) and interactions between stem cells and [membrane proteins](#). Manipulating cell seeding density is a common biological technique in controlling [cell-cell interactions](#), but controlling local density is difficult and it is often difficult to decouple effects between soluble signals in the medium and physical cell-cell interactions. Micropatterning of cell adhesion proteins can be used in defining the spatial positions of different cells on a substrate to study human ESC proliferation. Seeding stem cells into [PDMS](#) microwells and flipping them onto a substrate or another cell layer is a method of achieving precise spatial control. [Gap junction](#) communications has also been studied using microfluidics whereby negative pressure generated by fluid flow in side channels flanking a central channel traps pairs of cells that are in direct contact or separated by a small gap. However, in general, the non-zero motility and short [cell cycle](#) time of stem cells often disrupt the spatial organization imposed by these microtechnologies.

Embryoid body formation and organization



Murine embryoid bodies in suspension culture after 24 hours of formation from embryonic stem cells.

[Embryoid bodies](#) are a common *in vitro* [pluripotency](#) test for stem cells and their size needs to be controlled to induce directed differentiation to specific lineages. High throughput formation of uniform sized embryoid bodies with microwells and microfluidics allows easy retrieval and more importantly, scale up for clinical contexts. Actively controlling [embryoid body](#) cell organization and architecture can also direct stem cell differentiation using microfluidic gradients of [endoderm](#)-, [mesoderm](#)- and [ectoderm](#)-inducing factors, as well as self-renewal factors.

Assisted reproductive technologies

[Assisted reproductive technologies](#) help to treat [infertility](#) and [genetically](#) improve livestock. However, the efficiency of these technologies in [cryopreservation](#) and the *in vitro* production of mammalian [embryos](#) is low. [Microfluidics](#) have been applied in these technologies to better mimic the *in vivo* microenvironment with patterned topographic and biochemical surfaces for controlled spatiotemporal cell adhesion, as well as minimization of dead volumes. Micropumps and microvalves can automate tedious fluid-dispensing procedures and various [sensors](#) can be integrated for real-time [quality control](#). Bio-MEMS devices have been developed to evaluate [sperm motility](#), perform [sperm](#) selection, as well as prevent [polyspermy](#) in [in-vitro fertilization](#).

Bio-MEMS in medical implants and surgery

Implantable microelectrodes

The goal of implantable [microelectrodes](#) is to interface with the body's [nervous system](#) for recording and sending [bioelectrical](#) signals to study disease, improve [prostheses](#), and [monitor clinical parameters](#). [Microfabrication](#) has led to the development of Michigan probes and the [Utah electrode array](#), which have increased electrodes per unit volume, while addressing problems of thick [substrates](#) causing damage during implantation and triggering [foreign-body reaction](#) and electrode [encapsulation](#) via silicon and metals in the electrodes. Michigan probes have been used in large-scale recordings and network analysis of neuronal assemblies, and the Utah electrode array has been used as a [brain-computer interface](#) for the paralyzed. Extracellular microelectrodes have been patterned onto an inflatable helix-shaped plastic in

[cochlear implants](#) to improve deeper insertion and better electrode-tissue contact for transduction of high-fidelity sounds. Integrating microelectronics onto thin, flexible substrates has led to the development of a cardiac patch that adheres to the curvilinear surface of the [heart](#) by [surface tension](#) alone for measuring cardiac [electrophysiology](#), and electronic tattoos for measuring skin [temperature](#) and [bioelectricity](#). Wireless recording of electrophysiological signals is possible through addition of a [piezocrystal](#) to a circuit of two recording electrodes and a single transistor on an implanted micro-device. An external transducer emits pulses of [ultrasonic energy](#) which impinge on the piezocrystal, and extracellular voltage changes are backscattered ultrasonically by the piezocrystal, allowing for measurement. A network of so-called "neural dust" nodes can map signals throughout a region of the body where the micro-sensors are implanted.

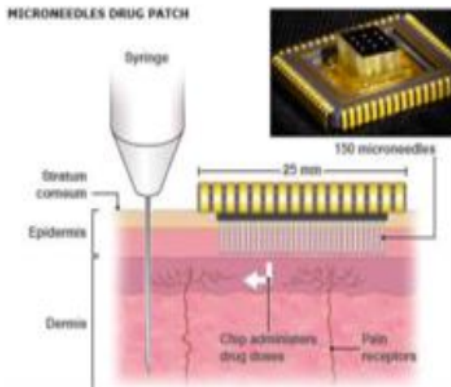
Microtools for surgery



A cardiac [balloon catheter](#) with temperature sensors, [electrocardiography](#) sensors, and [LEDs](#) is a surgical bio-MEMS.

Bio-MEMS for [surgical applications](#) can improve existing functionality, add new capabilities for surgeons to develop new techniques and procedures, and improve surgical outcomes by lowering risk and providing real-time feedback during the operation. Micromachined surgical tools such as tiny [forceps](#), microneedle arrays and tissue [debriders](#) have been made possible by metal and [ceramic](#) layer-by-layer microfabrication techniques for [minimally invasive surgery](#) and [robotic surgery](#). Incorporation of [sensors](#) onto surgical tools also allows tactile feedback for the surgeon, identification of [tissue](#) type via strain and density during cutting operations, and diagnostic [catheterization](#) to measure [blood flows](#), pressures, [temperatures](#), [oxygen](#) content, and chemical concentrations.

Drug delivery



Transdermal microneedles patch is less invasive compared to conventional drug delivery by hypodermic needle.

Microneedles, formulation systems, and implantable systems are bio-MEMS applicable to drug delivery. Microneedles of approximately $100\mu\text{m}$ can penetrate the skin barrier and deliver drugs to the underlying cells and interstitial fluid with reduced tissue damage, reduced pain, and no bleeding. Microneedles can also be integrated with microfluidics for automated drug loading or multiplexing. From the user standpoint, microneedles can be incorporated into a patch format for self-administration, and do not constitute a sharp waste biohazard (if the material is polymeric). Drug delivery by microneedles include coating the surface with therapeutic agents, loading drugs into porous or hollow microneedles, or fabricating the microneedles with drug and coating matrix for maximum drug loading. Microneedles for interstitial fluid extraction, blood extraction, and gene delivery are also being developed. The efficiency of microneedle drug delivery remains a challenge because it is difficult to ascertain if the microneedles effectively penetrated the skin. Some drugs, such as diazepam, are poorly soluble and need to be aerosolized immediately prior to intranasal administration. Bio-MEMS technology using piezoelectric transducers to liquid reservoirs can be used in these circumstances to generate narrow size distribution of aerosols for better drug delivery. Implantable drug delivery systems have also been developed to administer therapeutic agents that have poor bioavailability or require localized release and exposure at a target site. Examples include a PDMS microfluidic device implanted under the conjunctiva for drug delivery to the eye to treat ocular diseases and microchips with gold-capped drug reservoirs for osteoporosis. In implantable bio-MEMS for drug delivery, it is important to consider device rupture and dose dumping, fibrous encapsulation of the device, and device explantation. Most drugs also need to be delivered in relatively large quantities (milliliters or even greater), which makes implantable bio-MEMS drug delivery challenging due to their limited drug-holding capacity.