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Biosensors and BioMEMS: A Survey of the Present Field

Biosensors and BioMEMS: A Survey of the Present Field

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Administrative Note

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Introduction

Biomedical sensors and implantable devices employing micro-electromechanical technology will be important to the future of biomedicine. Once just science fiction, the notion of a bionic man is now becoming a reality through the application of microscale technologies. Today it is not unusual to have a friend or family member who has an implantable device, such as a pacemaker, defibrillator, cochlear implant, biosensor, neurostimulator, or insulin pump.

We have only seen the beginning of this technology development, and today's state-of-the-art implantable devices will be seen as crude and cumbersome tomorrow. With the rapid pace of development, it is probable that within the next two decades we will have as many medical treatments based on microdevices as there are pharmaceutical solutions today. This paper examines some of the most recent advances in the field, as well as the technologies that are likely to appear in the next few years.

What is BioMEMS?

BioMEMS stands for biomedical micro-electro-mechanical systems. It is a name applied to biological and medical devices that are created using advanced fabrication processes that allow the devices to be very small relative to comparable devices produced by traditional techniques. BioMEMS devices can also exploit the microscale to provide new functions that are not practical or possible in large-scale devices.

The name applies to an exceptionally wide variety of engineered devices that derive from electrical, mechanical, chemical, and molecular engineering. The name distinguishes these from nanoMEMS which are submicron in scale such as carbon nanotube structures.

Recently BioMEMS has become something of a misnomer as many of the latest technologies are being designed and developed based on nanoscale technologies which are many times smaller than microscale technologies. While current devices are manufactured mostly on the microscale, many of the functioning parts and the materials they operate on are at the nanoscale level.

NanoMEMS for biomedical applications are mostly carbon-based materials that have emerged as prime materials because of their favorable mechanical and electrical properties. Carbon-based nanostructures such as graphene exhibit a high Young's modulus (stiffness), high strength, low density, low friction and large surface area. The low friction of a carbon nanotube allows production of practically frictionless bearings and has thus been a huge motivation towards applications such as nanomotors. Carbon nanostructures are much stronger than steel, which allows carbon-based materials to meet high-stress demands in biomedical applications such as weight-bearing prosthetics (like hip-joint or bone replacements), where other materials would fail.

The field of BioMEMS encompasses micro devices that are often but not exclusively made by the same photolithographic techniques used to make computer chips. Their applications include neuroprosthetics, sensors and actuators, and microchemistry systems. A microchemistry system, often called a lab on a chip, can analyze chemical properties of a very small quantity of material such as a tiny blood sample. Advanced systems can perform several tests on the sample at one time.

There are also drug-delivery systems, miniature hearing aids, artificial retinas, DNA analysis systems, cancer diagnostics, and an amazing variety of devices which support the function of the human body. Figure 1 shows some devices that were developed by the faculty of Biomedical Engineering at Arizona State University.

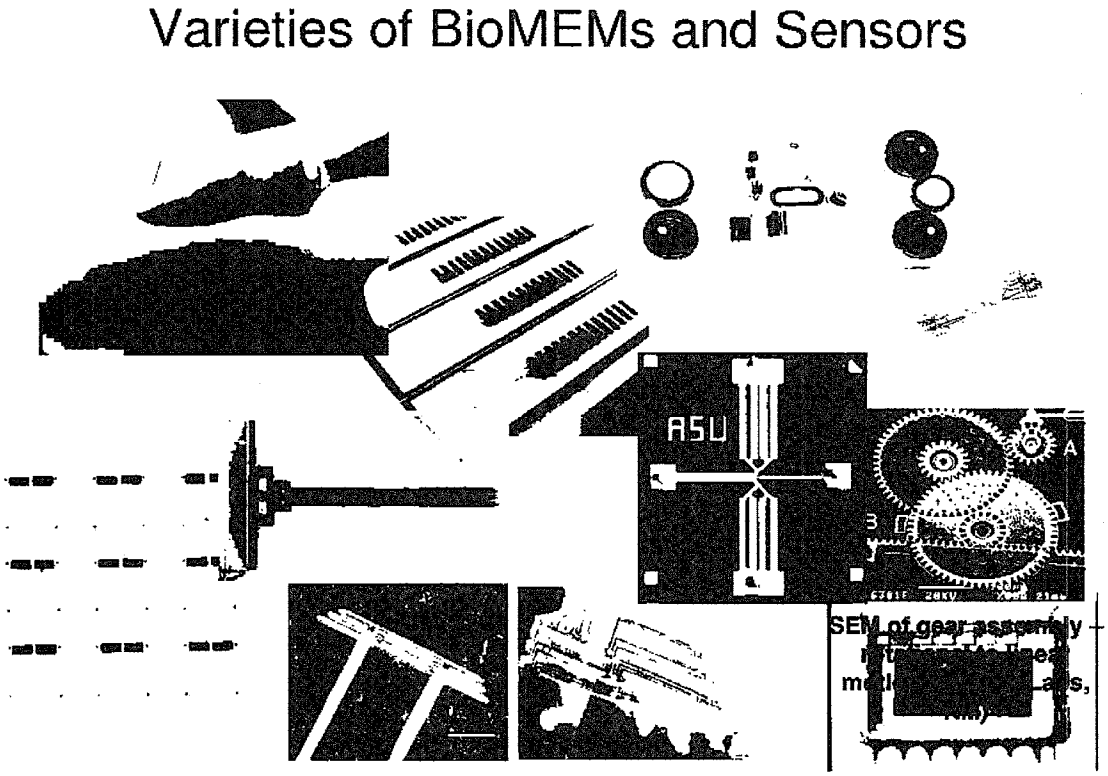


Figure 1. Varieties of BioMEMS and Sensors. These BioMEMS devices were made at Arizona State University (ASU). They represent many of the areas of BioMEMS research.

CHARACTERISTICS OF BIOMEMS

Conceiving and designing BioMEMS devices requires a different perspective of the physical world. These devices can operate on principles such as capillary force, *van der Waals* forces, and electric-field forces. These forces become relatively strong when the size scale reaches very small dimensions. In the realm of the very small, the force of gravity is far less important than electrical charge and viscosity.

From the perspective of microscale devices, engineers need to think about accomplishing tasks on an extremely subtle scale but with an exceptionally effective result. BioMEMS employs engineering sciences in ways that are more than just scaling down familiar devices used in biomedical applications. Rather, employment of new structures and new materials including polymers and biological components is necessary. In addition we need to be concerned about things like biocompatibility (the effect of the device on body tissue) as well as the degradation effects of tissues and body fluids on the device itself.

Development of a new product that is targeted for implantation into the human body is particularly expensive due to federal regulation of medical devices. Devices must be shown to be *effective* for their intended use, and above all devices must be proved *safe*. Bringing a new device to market is not unlike the development of a new drug. In order

to gain federal approval there may be biocompatibility issues to resolve, clinical trials to perform, and Food and Drug Administration (FDA) requirements to satisfy.

CATEGORIES OF BIOMEMS

We divide the BioMEMS field into several subcategories. Some devices are targeted to be implanted into the human body or are applied to the body. Many of these are being developed as *alternatives to drugs* which therapeutically treat illness or disease. There is also activity in using microscale stimulation devices to treat neurological disorders such as epilepsy and Parkinson's disease and to treat neurological injuries such as stroke or trauma to the spinal cord.

Another promising field of research is directed towards integration of the human brain with microelectrical components. For example implants are being developed which allow volitional (thought) control of machines. Most of this effort is targeted towards rehabilitation of individuals who are quadriplegics in order to allow them some control over their environment.

Then there are devices being developed for clinical applications such as rapid blood analysis, and there are those targeted for benchtop biological research. In this review, the focus is on specific microscale and MEMS-based devices that are used in medicine and biology.

These include:

- Micromachines that interface to brain electrodes.
- Blood glucose sensors.
- Microfluidics.
- Neural Interfaces.
- Neurostimulators.
- Microbeam sensors.

Several of these technologies have been the subject of research by the present author and by colleagues at Arizona State University.

BioMEMS Micromachines

Micromachines made by photolithography are among the most complex and sophisticated of all MEMS devices. They have seen some application in various forms of biomedical devices where motion must be achieved with an implantable device. Motion within the human body by a device is difficult since it implies sliding surfaces, a need for electrical power, and long term reliability and stability. Biomedical implants are usually introduced by surgery and so once implanted cannot be easily removed for servicing.

Only a few BioMEMS applications are well known for implanted devices and these are used for positioning and repositioning of sensors. A specific example is application to brain-electrode systems.

BRAIN-IMPLANTED BIOMEMS MICROMACHINE NEUROELECTRODES

There are applications in biomedicine and research where very small electrodes are implanted into the brain. These microelectrodes are used in the cortex (surface layer) to detect electrical activity associated with the volitional desire to move some part of the body. Electrical activity is recorded as a very small change in voltage within the tissue.

Near the top of the human head and about 2 cm beneath the scalp lie the parts of the brain where nerve cells (neurons) are found which control the muscles of the body. Specific locations in the cortex are associated with specific parts of the body. When a person moves a limb, there can be detected a corresponding electrical activity of these neurons. Neuroscientists have recorded these signals and developed a kind of map of the brain that defines what brain cells actuate certain muscles of the body.

An interesting phenomenon occurs in which some of these brain cells become active even when there is intent to move a limb but no actual movement occurs. In a healthy person the intent to move can be detected by measurement of microvolt signals from the brain cells about 120 milliseconds before any muscle movement occurs. In a person who has lost a limb or has become paralyzed, the intent to move can still be detected in the brain even though there is no limb movement.

Thus in principle there is an ability to electrically record from the brain and determine a person's intent to move. Monitoring of the brain is accomplished by using an array of implanted microelectrodes whose signals anticipate movement of specific limbs. These signals can in turn be used to control machines. This is the idea behind advanced devices that allow quadriplegics to interact with their environment. Signals from implanted electrodes are used by computers to control robotic actuators.

A problem recording the signals occurs because the body naturally tends to encapsulate the electrodes with scar tissue, meaning the electrodes lose electrical contact with the neurons. A solution to this problem incorporates a very small electromechanical actuator attached to the electrode. This device allows the implanted electrodes to change their position occasionally in order to continue to monitor neural events. By moving the thin rod-like electrode up or down a small distance after several months of implantation, the useful lifetime of the electrode array can be greatly extended.

The BioMEMS device described here is meant to be implanted under the skull and on top of the brain cortex. It allows fine adjustment of less than a millimeter in order to make sure the electrode system, once implanted, is able to contact the desired brain cells even after the electrode has been encapsulated by scar tissue over time.

Figure 2 shows a photomicrograph of a gear-driven micromachine that was made through collaboration between ASU and Sandia National Laboratories. The device is driven by an electrostatic vibrating comb motor. Under a microscope it can be seen that the combs move in an oscillatory fashion at about 40 Hz when energized. Electrostatic forces between two blade-like combs a few tens of microns apart are operative with about 15 volts as the electric field source.

The Neural Probe chip enables precise bi-directional positioning of the microelectrodes in the brain with a step resolution in the order of 8.8 μm. The thermal microactuators allow for a movement of the microelectrodes of up to 5 mm in either direction making it suitable for positioning microelectrodes in deep structures of a rodent brain.

The rest of the mechanism converts an oscillatory motion to a linear motion through a ratchet-type configuration. Figure 3 illustrates the basic unit where there are two comb-like arrays on either side. These generate the actual force. They are connected to a cross piece that converts oscillatory motion in concert with a spring to a pulsatile linear motion of the vertical shaft. This connects to some gears which do a mechanical transformation.

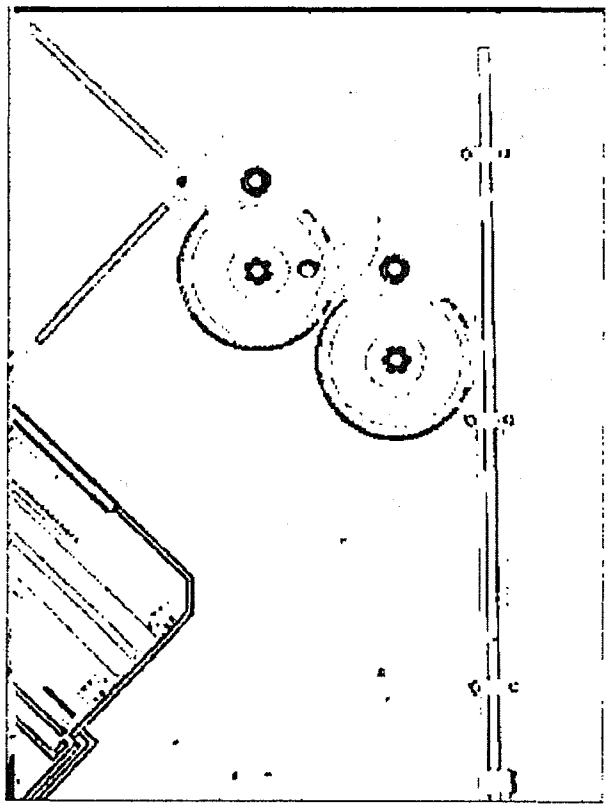


Figure 2. Functional MEMS Micromachines Attached to Sensing Bioelectrodes. The gears have features as small as 50 microns, less than the size of a period on a printed page. (Courtesy of Dr. J. Muthuswamy, Bioengineering Department, ASU)

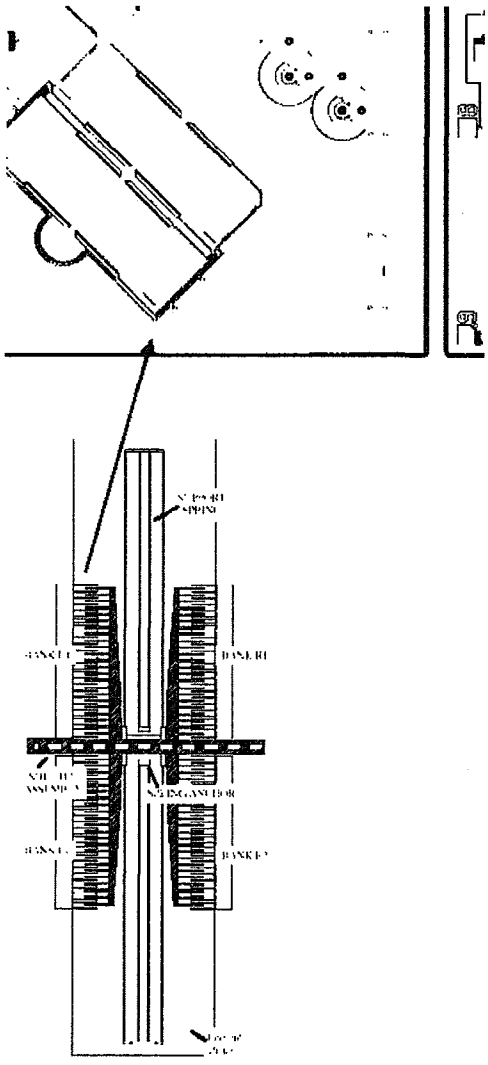


Figure 3. Schematic Diagram of a MEMS Motor Complete With a Method of Converting Oscillatory Motion to Linear Motion. (Courtesy of Dr. J. Muthuswamy, Bioengineering Department, ASU)

Figure 4 illustrates the basic physics where the force is generated. A voltage V, is applied to the horizontal sliding shaft. There arises an electrical field (shown as the curved arrow) that tends to pull the shaft into the cavity between the two outer combs. This is the basic motive force of the device. The force generated by each comb is very small so there are constructed many similar structures that form a comb-array. All of the forces act in parallel.

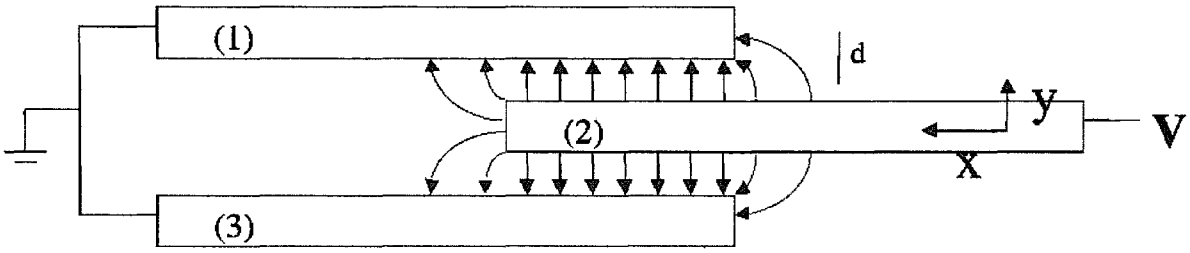


Figure 4. Illustration of the Basic Physical Principle of the BioMEMS Motor (Muthuswamy et al.)

The device is fabricated by using Sandia's Ultraplanar Multi-level MEMS Technology (SUMMiTV) process, a 5-layer polysilicon micromachining technology. The layers are chemically sensitive to different processes and can be selectively removed by etching with different reagents.

For example silicon can be removed using an etchant which preferentially removes silicon only along certain crystalline planes. Other layers can be undermined through the use of acids. The components of the micromachine are patterned on the silicon using photoresist masks. A mask is a covering which prevents parts of the silicon from being etched away. Figure 5 gives an idea of the sophistication of this MEMS fabrication technique.

The end result is that the long rod at the right of the picture in Figure 3 moves up and down with a speed of about 1-2 mm per second. The speed can be adjusted through the number of teeth designed on the reduction gears.

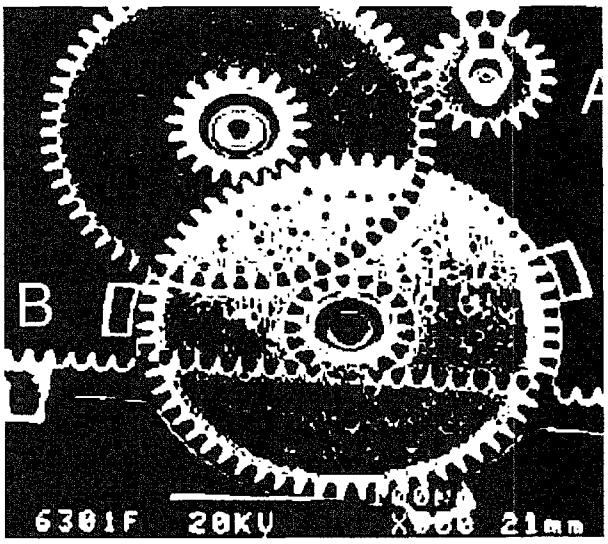


Figure 5. Close-Up Views of the Micro-Motor Gears (Sandia National Labs)

Single unit recordings were obtained from the somatosensory cortex of adult rats over a period of three days demonstrating the feasibility of this technology. This device has been implanted in rats and its development supported by the neuroprostheses program of the US National Institutes of Health.

Fluidic BioMEMS

The application of microscale fabrication techniques has allowed the manipulation of very small volumes of fluids on the nanoliter and even picoliter scales. This allows

chemical processes such as separations, reactions, and analysis to be conducted with very small amounts of sample.

According to a forecast by the Nexus Task Force, the market for BioMEMS is expected to reach $18 Billion in 2005. The commercial success of these devices and the technical potential of other BioMEMS has driven research in a number of areas. As a result, over the past few years, fluidic BioMEMS devices have become the largest and most diverse applications of MEMS devices.

Fluidic BioMEMS now include:

- Drug-delivering neuroprobes.
- Biosensors (general).
- Bioreactors.
- Cell-handling devices.
- Drug-delivery devices.
- Micro-chromatography systems.
- Microfluidics.
- Molecular detection/handling.
- Neural interface devices.
- Optical/retinal sensing.
- Surgical devices.
- Tissue-handling devices.

There are obviously application overlaps within these devices and some are integrated with others to create system-level or multi-sensing devices. Applications run the gamut of the imagination including identification of bacterial or viral agents, drug testing, home testing, environmental safety and security, and drug-delivery technologies.

DRUG-DELIVERY PUMPS

Drug delivery to the human body for medical therapeutic purposes has long been by swallowing substances or by injection. However this approach creates rapid rises and falls in drug concentration in the blood stream because the dose is typically introduced all at one time. The blood titer of a drug may thus peak at undesirably high levels in an effort to sustain the drug action over a longer period of time.

Treatment of many medical conditions, such as cancer by chemotherapeutic drugs would best be accomplished by a steady and long sustained infusion of the drug into the blood. This would keep the tumor-fighting drug at some optimal concentration and so make it maximally effective. High levels of chemotherapeutic drug levels in the blood are toxic and levels that are below some threshold are ineffective.

Methods of creating a more sustained blood concentration of a given drug are desirable to increase drug effectiveness. Similarly there are other medical applications for controlled-release drug-delivery systems, such as that for insulin, where it is desirable to maintain a more sustained dose over a period of hours.

There has been the application of MEMS technologies in the creation of better ways to deliver drugs to the human body by way of small implantable reservoirs that can slowly release their drug over a prolonged period of time. Figure 6 shows a MEMS-based insulin-delivery pump for the treatment of diabetes. This is perhaps one of the largest applications of BioMEMS in medicine.

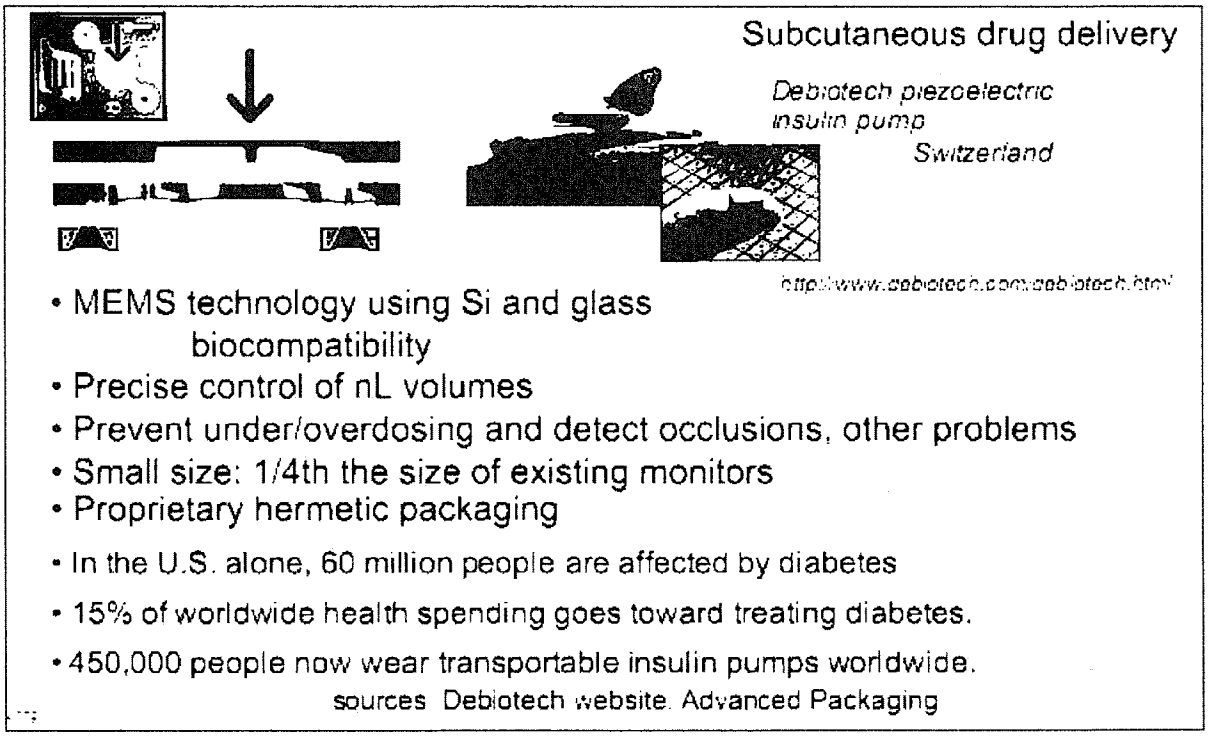


Figure 6. An Insulin MEMS Pump. An implantable drug-delivery device.

Diabetes is a disorder in which glucose (blood sugar) is not properly taken into the cells of the body. This process is normally mediated by the hormone insulin, which is produced by the islet cells of the pancreas. Insulin circulates in the blood to actuate a receptor on tissue cells that causes them to uptake glucose from the blood stream. If there is too little insulin (as in Type 1 diabetes) the cells will not uptake glucose from the blood, the cells will starve, and blood sugar levels become too high from unabsorbed glucose. Alternatively, cells may not respond properly to normal insulin levels (as in Type 2 diabetes) and require artificially higher levels of insulin to uptake glucose. This is called insulin resistance. In either case, blood insulin levels can be increased by using insulin injection devices.

Brittle diabetes is a condition where blood glucose levels fluctuate wildly with eating or fasting causing hyperglycemia (too high a blood glucose—more than about 100 mg percent) or too low (hypoglycemia—less than about 60 mg percent). Brittle diabetics

have a significant problem in controlling their blood sugar and sometimes insulin doses are not adjusted finely enough or frequently enough to maintain the blood glucose at healthy levels. This form of diabetes is responsible for fainting (ketoacidosis) and other serious symptoms such as poor blood circulation to the limbs. Poor circulation can result in diabetic ulcers and may sometimes necessitate amputation of the limb.

Insulin pumps are often worn by brittle diabetics because a slow infusion of insulin works better in stabilizing blood glucose levels rather than periodic injections. A belt-worn insulin-delivery system looks something like an old-style audio player. These systems are typically controlled by a small screw-type pump powered by batteries. The reservoir carries several milliliters of insulin for dispensing over an extended period of time. The injection needle is connected (underneath the clothes) to a catheter and then to the pump. The systems are reasonably effective but are cumbersome and require the needle to continuously reside subcutaneously in the abdomen.

A MEMS *implanted* insulin pump is a less cumbersome and perhaps more convenient means of slowly infusing insulin at a programmed rate. The implanted device is refilled periodically by introducing a needle through the skin and tissue to a septum in the device.

The MEMS insulin pump shown in Figure 6 is surgically placed under the skin. It has a rubber septum on the top for filling with insulin. This implementation has a piezoelectric element that moves in response to electrical charge. When actuated by a timer the element oscillates, creating a pressure inside the device that dispenses insulin. The device is programmable for dispensing at various rates.

Although the implantable MEMS pump was developed for diabetes, the pump has application to the slow measured delivery of many other drugs including 5-fluoruracil used for cancer therapy and theophylline for treatment of asthma.

What is a Biosensor?

A primary application of BioMEMS is in the creation of sensors for blood chemistry and other biophysical parameters of the human body.

We can define a Biosensor as:

- A sensor whose application is primarily in the measurement of quantities within a biological system, such as chemical, electrical, and physical parameters.
- A sensor *incorporating* a biological component (enzymes, living cells, antibodies) typically used to measure chemical concentration. This definition does not require that the sensor be deployed *within* a biological system.

The two definitions which are somewhat different have their origin with different influential investigators who wrote textbooks in the early days of this field. The latter definition is prevalent in Europe.

A biosensor is normally constructed by immobilizing a biologically active material (such as an enzyme) onto an electrical sensor that measures a fundamental physical quantity like electrical current, voltage, mechanical strain, temperature, or frequency. The specific sensor material is chosen because it reacts to a desired measurand (such as

concentration of glucose) and thus causes a change in one of these fundamental quantities. Electrical sensors can be inherently small and so we have a combination device with biology and electrical sensing in a small and compact form.

When compared to much larger bench top machines, microscale configurations of sensors usually have a superior performance because they are less prone to various interferences such as power-line noise and they have much shorter diffusion distances for sensed molecules. Some physical quantities such as micro-degrees of temperature change are much easier to measure over small distances.

BioMEMS Implantable Sensors

Biosensors placed inside the body, or *in-vivo,* measure biological parameters such as blood pH, oxygen, carbon dioxide, and blood glucose. (Blood pH is a measure of the acidity of the blood.) These parameters are the most important in medicine since these are all independent blood chemistries that give a moment-to-moment insight into the physiological state of a living being.

Since the early days of the space program NASA has been interested in ways of noninvasively monitoring these blood parameters in astronauts for the instant assessment of their physiological condition. Until recently, blood withdrawal was the only accurate and reliable way to obtain such information. Indwelling sensors that use needle penetrations are now available but still have trouble with accuracy and longevity.

The military has also been interested in the assessment of the state of readiness of a soldier which is reflected in his blood chemistry. An exhausted solider will show a highly acidic blood pH (less than about 7.3). Remote electronic readout of biosensor information of a soldier to a central command center is presently the stuff of science fiction movies but reflects real desires of the military.

Figure 7 shows a photo of a pH sensor (which measures concentration of hydrogen ions). It is designed as a needle for tissue insertion or placement at the end of a catheter for introduction into the blood stream. The pointed tip is inserted into the medium to be measured, and the electrical signal and power supply are connected to the device at the gold film contacts at the base.

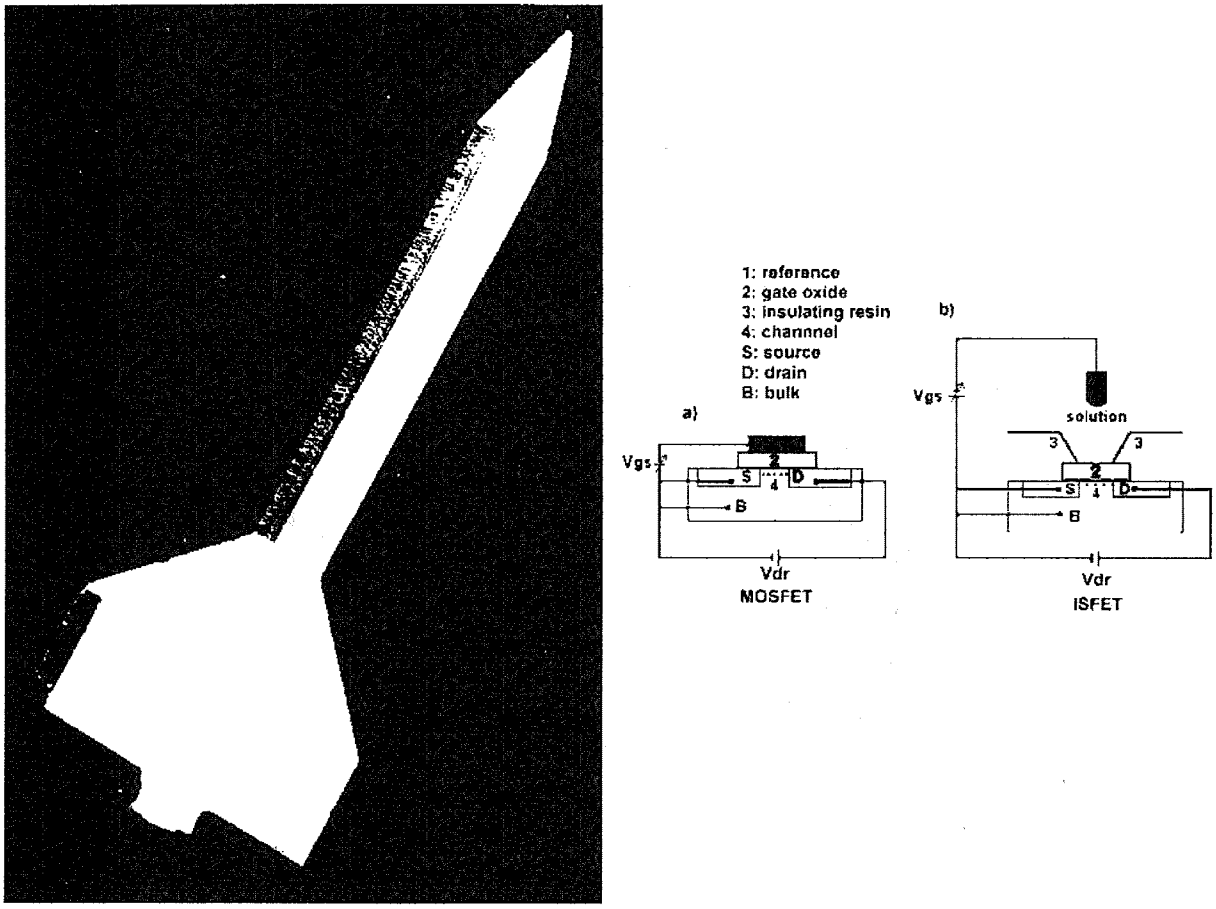


Figure 7. An ISFET (Ion Sensitive Field Effect Transistor) Needle-Type pH Sensor for Monitoring Tissue Physiologic Status (http://www.ee.seikei.ac.jp/~seiichi/lecture/Biomedical/09/09-biosensor.html)

The sensor element is an ion sensitive field effect transistor (ISFET). This device is micromachined from silicon by using standard semiconductor photolithography processes. The actual active sensor surface is near the tip of the device where there is a thin film of silicon nitride covering the gate region of the transistor. The gate is sensitive to very small changes in electric field, and these in turn cause relatively large changes (*or gain*) in the current flow through the transistor.

At the center in Figure 7 is a standard transistor diagram showing the gate and its relation to the other contacts. On the right is shown the *pH* sensitive ISFET system that measures an electric field change across the nitride membrane. The membrane is placed directly in contact with the liquid to be measured. It is not known the exact process that occurs at the membrane that gives rise to electric field shifts on the gate. Presumably hydrogen ions reversibly adsorb onto the interface between the nitride membrane and the solution causing local electric field changes on the gate.

This device is not meant for permanent implantation but rather short term applications in research or medical surgery where the sensor is used over just a few hours. Proteins and other biological molecules adhere to the gate region of the ISFET and cause a slow

drift and offset in the reading and so shifting the calibration. Depending on its exact construction some versions however can be effective for as much as a few days.

Figure 8 shows the latest in microdevice oxygen sensors. (This particular version is not specifically a MEMS device since it is not made by photolithography and it is neither electrical nor mechanical in nature.) It represents however the trend in oxygen sensors towards optical measurement techniques that are evolving towards optical systems based on MEMS. These sensors can be implemented in small sizes with optical fibers that transmit and receive light from the sensor surface at the tip. Variations on this basic principle are used for indwelling blood oxygen catheters during surgery.

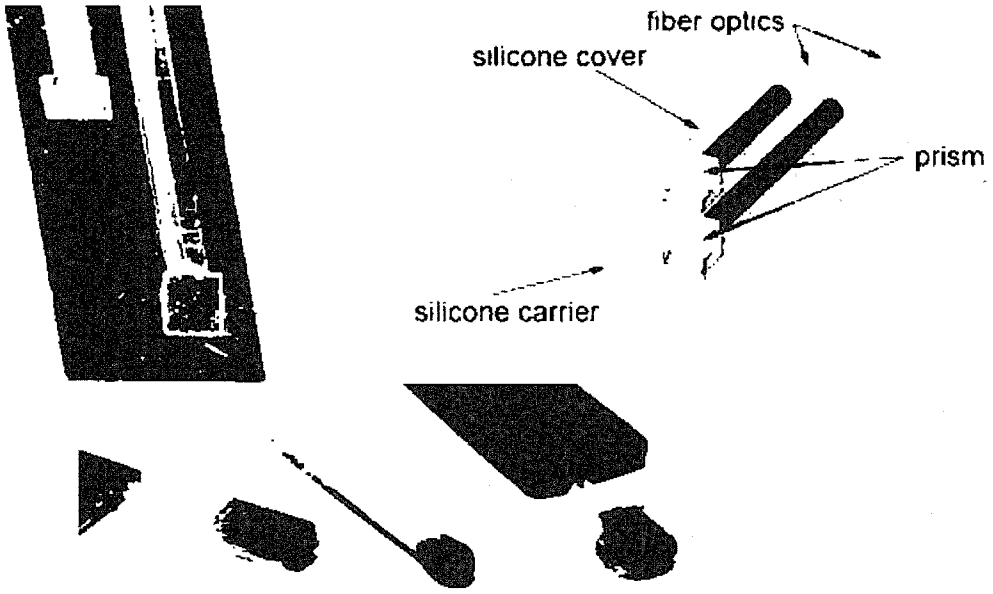


Figure 8. A Fiber Optical Oxygen Sensor Showing Two Optical Fibers Mated to Plastic Prisms That are Coated With an Oxygen-Sensor Coating, Usually a Fluorescent Ruthenium Compound
(http://www.imtek.de/content/projekte_en.php?ls=11)

The oxygen sensors respond to changes in absorption of oxygen into the special coating at the tip, or in some versions, they respond to fluorescence of a thin oxygen-sensitive coating placed on the prism surface. The secret to good performance lies in the proprietary selection of the specific chemical species selected for the coating.

Blue light is usually transmitted down the optical fiber and the coating glows orange in proportion to the amount of oxygen present. The light from the glow is conducted back toward the supporting instrumentation system. The intensity of the orange light then indicates oxygen levels in the solution. The particular sensor shown in Figure 8 uses two optical fibers. The one on the left side is not sensitive to oxygen, but rather acts as a reference system that is used to compensate for changes temperature, ambient light, and other system variables.

So far, despite decades of work, no continuous monitoring sensor that is self contained and implantable has been successful for long term usage. The human body is an exceptionally hostile environment for foreign materials. Even our most advanced biosensors fail over a period of days or weeks if continuously exposed to the body environment. This failure is mostly due to the sensor chemistry wearing out in one way or another or attack of the sensor interface by the body's immune system. Failure is not generally attributed to the materials of which the sensor is made.

MEMS Blood Glucose Sensors

Diabetes is an enormous world problem. A significant fraction of the annual world health care expenditures can be traced back to diabetes, and its cousin, obesity. Measurement of blood glucose on a regular basis, usually 3-4 times a day, allows a diabetic to regulate his diet and insulin dose to achieve normal glucose levels.

Perhaps one of the most needed biosensors is that for an *in-vivo* blood glucose sensor. These could be used to automatically control of the output of an insulin-delivery pump. This would constitute a major improvement in the treatment of diabetes by automatically using blood glucose concentration feedback to stabilize the blood glucose levels with the exact level of needed insulin. This kind of system is sometimes called an artificial endocrine pancreas since it mimics the normal function of the pancreas in regulating insulin release.

There are several attempts at using MEMS devices to produce indwelling glucose sensors. These are presently on the market, but currently the sensors have relatively short lifetimes, are disposable, and require replacement every few days.

Figure 9 shows this basic idea. An implantable sensor produces an electrical output that reports the blood glucose concentration. Its signal is transmitted to a receiver and then processed by a computer to drive a belt-worn pump.

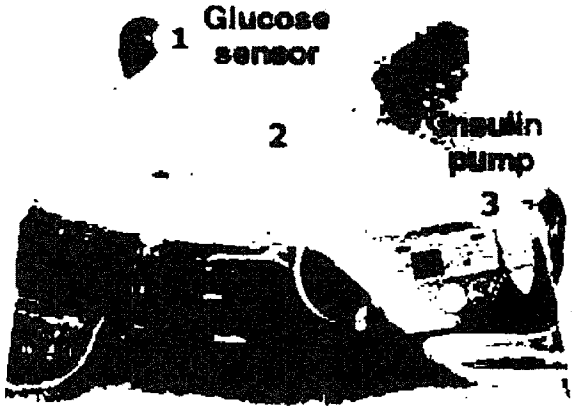


Figure 9. Control of an Insulin-Delivery System by an Implantable Glucose Sensor (Medtronic Inc.)

The creation of such a sensor has been a daunting problem from more than 30 years. The most recent attempts have been in the use of micromachining to produce optical devices of very small size and to produce wireless transmitting electrochemical sensor devices small enough to be injected into the body.

Coincident with the development of sensors has been the need to develop very small wireless telemetry systems that transmit the sensor data to outside the body.

COMMERCIAL BLOOD GLUCOSE SENSORS

The present method of blood glucose monitoring depends on a needle puncture of the skin to withdraw a drop of blood to place on a color changing test strip. The test strip is read by a small handheld reader. Glucose test strips do not have to necessarily be small, but by making the sensor very small the amount of blood required for the test is reduced. The sensors often are made by techniques of photolithography or in some cases by microscale screen printing in order to achieve reproducibility.

The skin-puncture test is painful and time consuming, and thus noninvasiveness is the key desired characteristic of glucose sensors. Research is being directed at a noninvasive glucose sensor that is accurate enough to work external to the body and through the skin. Short term (a few days) wearable needle glucose sensors are available from major companies like Medtronic Inc. but fall short of the convenience of a noninvasive sensor.

ENZYME-BASED BIOSENSORS

The key component in most biosensors is a reactive chemistry on the sensor surface. The sensor chemistry is chosen to give it specificity to only one analyte (such as glucose). The concentration of the analyte is determined by a sensor that can directly measure the analyte reaction products reacting with the sensor surface. For example sensor chemistries to measure glucose are often based on glucose oxidase enzyme which promotes a chemical reaction at the sensor surface. Glucose oxidase enzyme complex structure is seen in Figure 10.

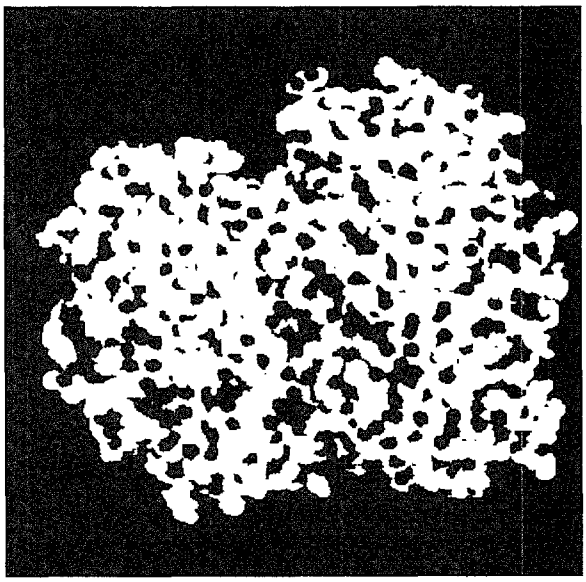


Figure 10. Glucose Oxidase Enzymes Like Glucose Oxidase are Large Folded Molecules That Act as Catalysts for a Chemical Reaction
(http://www.innovations-report.de/bilder_neu/17279_gluc.jpg)

Glucose oxidase catalyzes the reaction:

glucose + O_2 (glucose oxidase) → gluconolactone + H_2O_2 + heat (79 kJ/mole)

This reaction of glucose with oxygen (from the air) occurs in the presence of glucose oxidase enzyme. The enzyme itself is a catalyst to the reaction and so is not consumed. Rather it presents favorable conditions and ability to transfer electrons on its molecular structure for glucose and oxygen to come together to react. Figure 10 shows the enzyme structure.

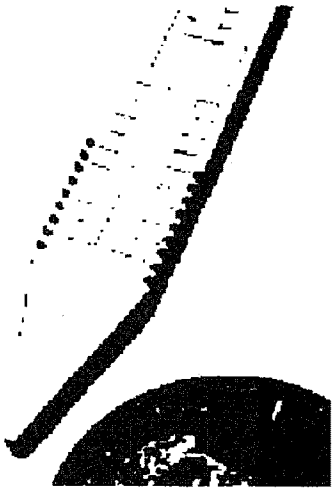
Typically in a sensor the enzyme is a large molecule and can be trapped in a porous gel and thus is not able to diffuse away from the sensor surface. Glucose and oxygen, being small molecules, can diffuse through the gel to the enzyme whereby the reaction occurs and the reaction products will diffuse away. The enzyme is unconsumed and the reaction process is continuous as long as glucose is present.

An enzyme-gel is applied in a thin layer over a electrically biased metal electrode. An electrical current flows through the electrode when the hydrogen peroxide produced by the glucose reaction is decomposed and causes an electrical current to flow. If there is a fixed concentration of glucose present, a corresponding amount of peroxide will be produced, and this can be measured by the electrical current flow. The amount of peroxide measured is an indicator of the glucose concentration. The actual amount of analyte consumed by a small BioMEMS sensor is exceedingly small and has no significant effect on the local concentration.

Figure 11 shows a MEMS implementation of a blood glucose sensor using this kind of approach (Advanced Biosensors Inc). The fine needle geometry is generally meant for insertion into tissue. This device is comprised of multiple independent small sensors and is actually a system of components that adds signal processing and interface electronics to allow its communication with insulin pumps for automated delivery of insulin based on patient need.

Advanced BioSensors blood glucose sensor

- Flip chip assembly
- ASICs, power sources and circuit components
- Separate sensors, mount with electrical connections, add the biocompatible polymer, assemble the sensor patch.
- The sensor based on glucose oxidase reaction with blood plasma from capillaries in the dermis.
- biochemical coating - sensor in place 3 - 7 days
- Docking port for electronics assembly with amplifier, ADC, wireless transceiver, power source
- encrypted digital data to wristwatch-sized monitor/recorder module
- In future combine the CGMS with an insulin pump to produce a closed-loop system—an artificial pancreas.



1 mm long x 200 um wide

Figure 11. An Experimental MEMS Blood Glucose Sensor

Glucose sensors based on electrical measurement of hydrogen peroxide products have been found to have the significant problem of a drifting baseline over time. It is difficult to maintain calibration of the sensors, and the electrode is affected by proteins and other substances in the blood stream. Although this sensing approach has had some success, its longevity in the body is much less than desired; sensors based on this approach decay after a few days of use.

THERMOPILE IMPLANTABLE GLUCOSE SENSORS

Towe et al. at Arizona State University have been working on an improved approach to blood glucose sensing. It depends on the fact that the glucose oxidase reaction generates a small amount of heat as indicated in the above chemical reaction. The

released chemical energy warms the surrounding environment to a degree that depends on the energy released in the exothermic reaction, which is characteristic of the particular chemistry and the amount of reactants. The heating is then dependent on the glucose concentration.

Figure 12 shows these devices. They are made by MEMS techniques involving the processes of using patterning by exposure to light through a mask (photolithography), metal deposition by evaporation, and sputter etching. The result is the fabrication of sensors in large arrays with many identical sensors on a single substrate. The figure is a photomicrograph of a part of a wafer production run with nine identical devices. Each one of the devices is less than 1 mm in size and would be cut apart and used independently. The darker rectangular areas at the top of each device are the electrical bonding pads where wires attach to the device to readout its electrical response.

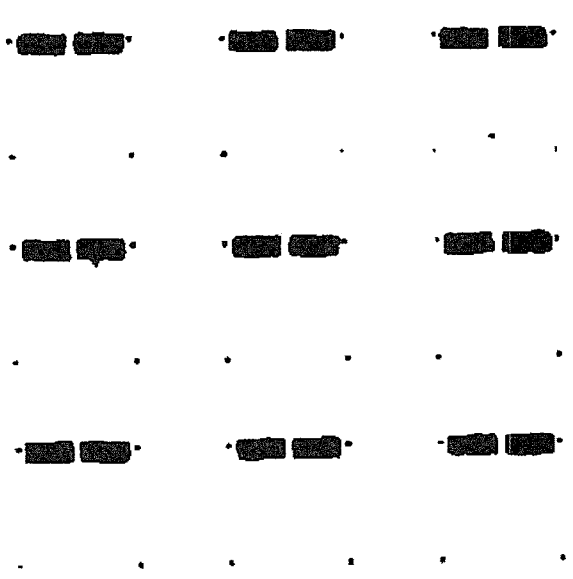


Figure 12. Photomicrograph 3 x 3 Array of Thermoelectric Glucose Sensors Constructed by Towe et al. at ASU

Figure 13 shows the basic process in the sensor operation. The sensor is planar and composed of a layered structure consisting of an enzyme gel and a thermoelectric temperature sensor. Glucose from the local medium naturally diffuses to the gel and the temperature sensor underlies a thin film of enzyme gel that contains the glucose oxidase. A second enzyme called catalase is used to secondarily break down hydrogen peroxide produced by the first reaction.

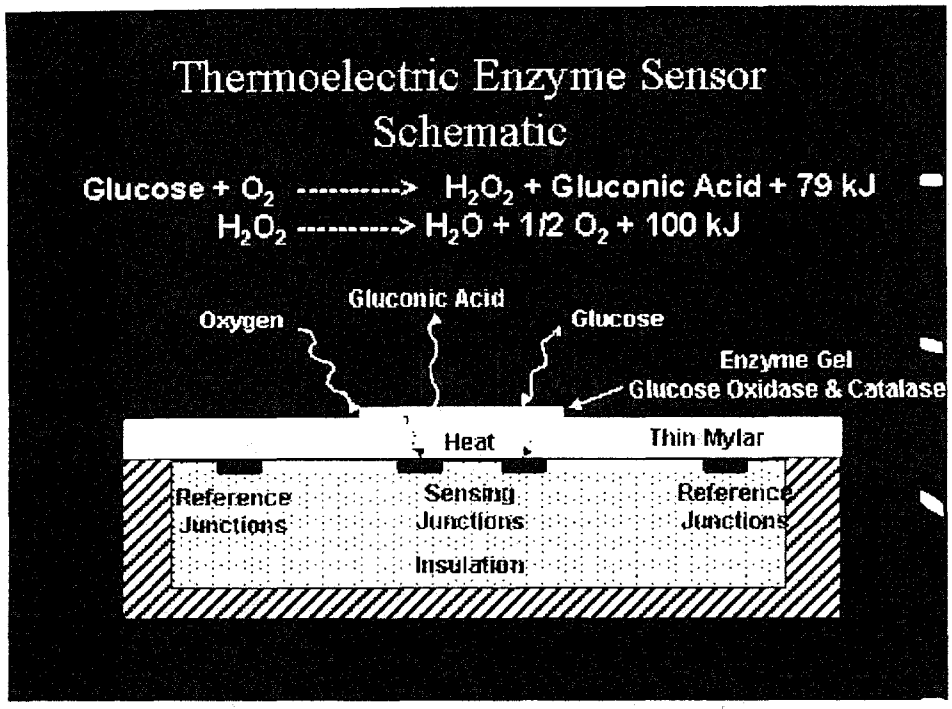


Figure 13. A MEMS Thermopile Glucose Sensor (Towe et al., Arizona State University)

Heat energy released in the gel is measured by the thermopile and is characterized in terms of kiloJoules (kJ) per mole of glucose consumed. At concentrations of glucose that are common in the blood stream, the temperature rise is very small, on the order of a hundredth of a degree (10 millidegrees).

Measuring this temperature change in the presence of ambient body heat or room-temperature changes of a few degrees is a daunting task, but can be done.

The measurement of the small reaction temperature in the face of possible ambient temperature shifts is accomplished by the use of a differential temperature measurement system.

This sensor employs what is known as the Seebeck Effect whereby dissimilar metals heated at their junction produce a voltage that is proportional to temperature. This concept is shown in Figure 14. This principle is then implemented as an array of metal junctions that when connected in series produce a higher output in order to detect smaller temperature changes.

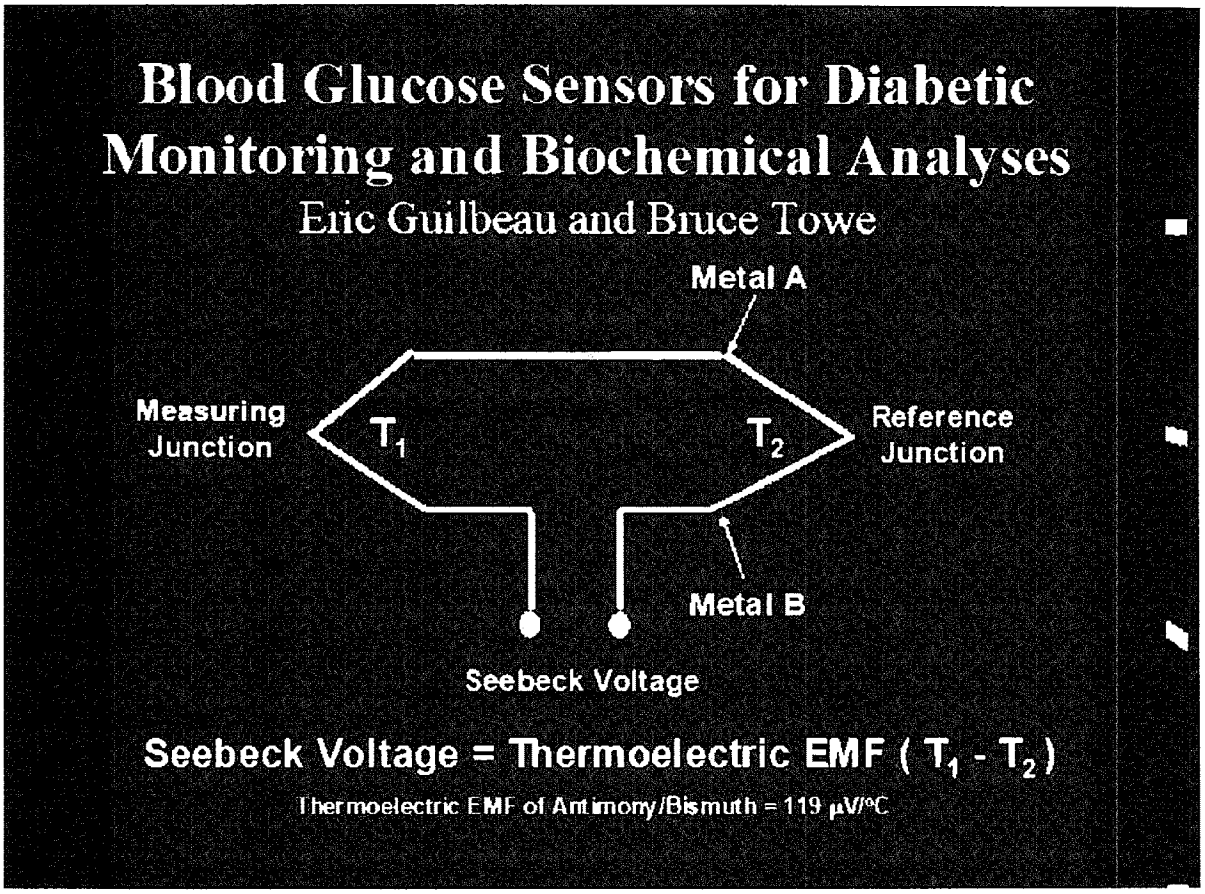


Figure 14. Illustration of the Thermocouple Principle (Guilbeau and Towe, ASU)

Figure 15 on the left panel shows a photomicrograph of a thermopile temperature sensor that is made from the metals antimony and bismuth. These metals are vacuum deposited as alternating thin films using a masking process to form horizontal lines of conductive metal. Each pair of lines has a sensing junction and a reference junction. When they are heated on their sensing junction with respect to their reference end (that is exposed to the ambient temperature), they generate a small electrical voltage.

Each junction is only a few tens of microns in size so it is possible to place dozens of junctions in series in order to increase the voltage signal. Figure 15 on the right panel shows that there are two banks of thermoelectric sensors with the central common junctions supporting an enzyme gel. Thermopiles are thin film temperature sensors and are differential in nature in that they measure temperature differences, and are thus sensitive enough to detect exceedingly small temperature rises.

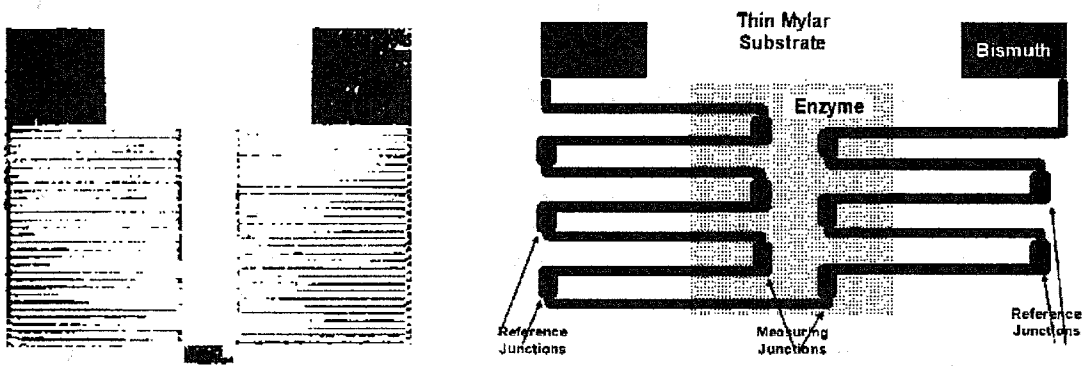


Figure 15. Thermopile Glucose Sensor (left) With Functional Illustration (right) are Made Using Photolithographic Techniques. This larger device is about 8 mm in length but has approximately 50 micron minimum geometries. (Towe et al.)

At ASU the glucose sensor has been constructed on a thin mylar plastic substrate and so can be curved into a cylinder to form a small tube. This tube is then sensitive to glucose concentrations on its outside exposure to the blood stream. Figure 16 shows a photograph and an illustration of a glucose sensor implemented in the form of a catheter. The electrical output signal is routed by wires down the length of the catheter for a remote readout. The device was implanted in a pig over a short duration and the decrease in blood glucose concentrations in response to administration of insulin were recorded and are shown in the right hand panel of Figure 16.

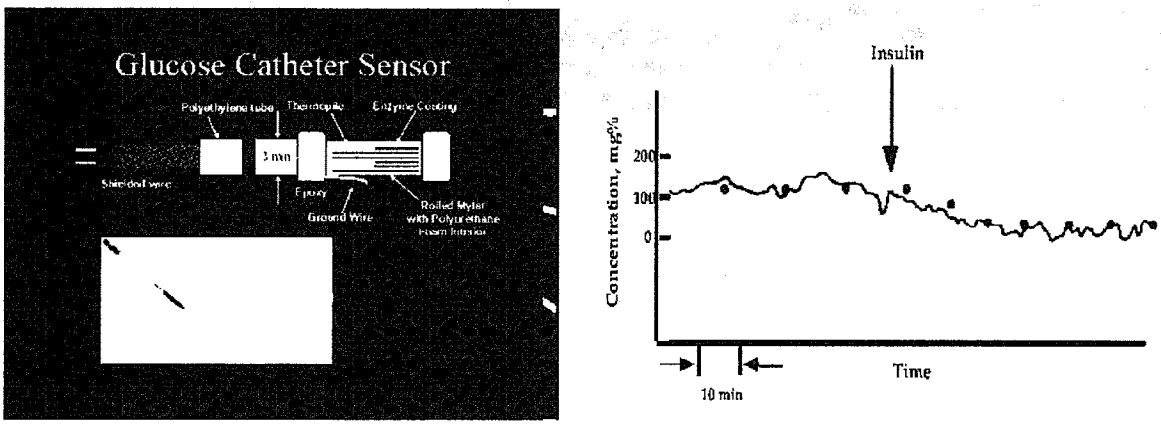


Figure 16. Photograph and Illustration of the Thermopile Glucose Sensor in a Catheter. Its configuration is shown (left) and its response in detecting changes in blood glucose in a pig as a result of insulin administration to the animal. (ASU research.) Towe et al.

This glucose sensor approach has been found to have limitations in stability when introduced into tissue or blood. Primarily the problems, as with many sensors, arise from the system chemistry and not as much from the electronic portion of the sensor. The glucose oxidase enzyme slowly decays over time and thus the sensor sensitivity and calibration drifts making it eventually unusable.

These problems have no easy solution. Investigators have been working with various forms of blood chemistry sensors for forty years or more. Microfabricated

thermoelectric sensors have improved reproducibility of the sensors, but there has only been limited success in applications of implantation into the body.

Neuroengineering by BioMEMS

BioMEMS types of devices, as we understand them today, were used first in neuroscience. There has been a long history of the study of the electrical nature of body tissue dating back to the days of Galvani and Volta. Frog nerves were found to be electrically stimulatable and the first recordings of bioelectrical events were accomplished very early with the invention of the string galvanometer.

More recently, pointed wires inserted into muscle, nerve and brain have given way to MEMS electrode systems that are made on silicon supporting substrates and the processes of photolithography used to define electrical current pathways. Figure 17 shows a modern electrode system for detecting and recording electrical signals from living things. Each of the square regions is an exposed film of platinum while the thinner conductors that contact the pads are insulated by a thin layer of a plastic and conduct the detected electrical signals to a connector system (not shown).

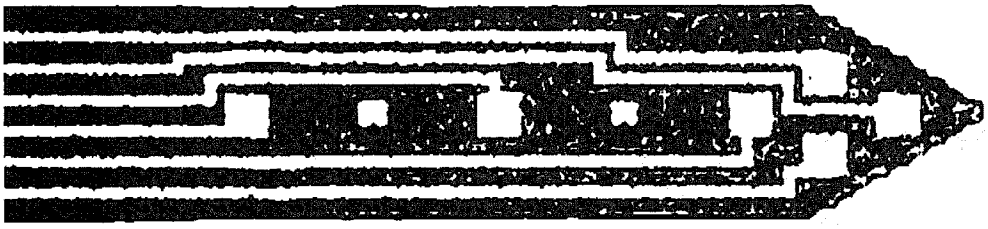


Figure 17. A MEMS Biopotential Electrode System. It is approximately 100 microns in width. Each square pad is an electrically sensitive region. (Wikipedia)

BIOELECTRIC EVENTS

The living processes of biology can in some ways be likened to that of a battery. The metabolism of life produces the charging of the battery while it discharges through a myriad of bioelectrical events that produce movement, thought, and cognition.

The transfer of electrical charge in the form of ions, mostly sodium, potassium and chloride is initiated by bioelectrically excitable cells of the nerves, muscles, and neurons of the brain. Collectively they constitute the wiring of the body. These ions result from the salts that are part of the composition of living things that have evolved from the sea.

The movements of these ions constitutes the flow of an electrical current in tissues and are called bioelectrical currents. These currents give rise to electrical potentials that can be detected from the skin or by biopotential electrodes inserted into organs or muscle.

Bioelectrical events are generated in tissues when there is an electrochemical change in the membranes of specialized cells of the brain and nervous systems.

Neuroelectrical devices interface to the brain and nervous systems through electrodes that touch excitable cell membrane and convert ionic current flows to electron flow in a wire. Conversely current flows in a wire are converted by electrodes to ionic flows in tissue.

The function of the entire human body is under control of the brain and the nervous system. So the use of electrodes of various types as a method of monitoring and controlling the function of the nervous system through bioelectrical currents and electrodes is potentially a very powerful method of treating in therapeutic ways. Figure 18 suggests the idea of an excitable cell of the brain having many tendrils or dendrites that extend outward to interconnect with other cells. Essentially these cells are the relay points of the bioelectrical wiring of the human body.

Figure 18. A Neural Cell Showing Dendritic Inter-Connections That are Electrically Active With Other Cells (artist's illustration)

An electrical interface to body tissues typically occurs through the use of microelectrode systems. These electrodes are often on the order of tens of microns to millimeter-order in sizes depending on their function. For example electrodes that simply measure bioelectrical activity of the nervous system can resemble needles insulated along their length having micron order tips both because they carry only small electrical currents and also because they need to be placed in very specific places and they contact just a single or few cells that control specific functions.

Bionics and Neurointerfaces

Neuroprosthetics (also called neural prosthetics) is a discipline related to neuroscience and biomedical engineering concerned with developing neural prostheses. Neural prostheses are devices that attempt to substitute for a motor, sensory or cognitive modality that might have been damaged as a result of an injury or a disease.

Bionics and neurointerfacing are relatively new concepts. A neurointerface is a combination of software and hardware that translates electrode signals to something which is understood by an electronic system. The term bionics is used when we connect organic matter with something artificial (human-made).

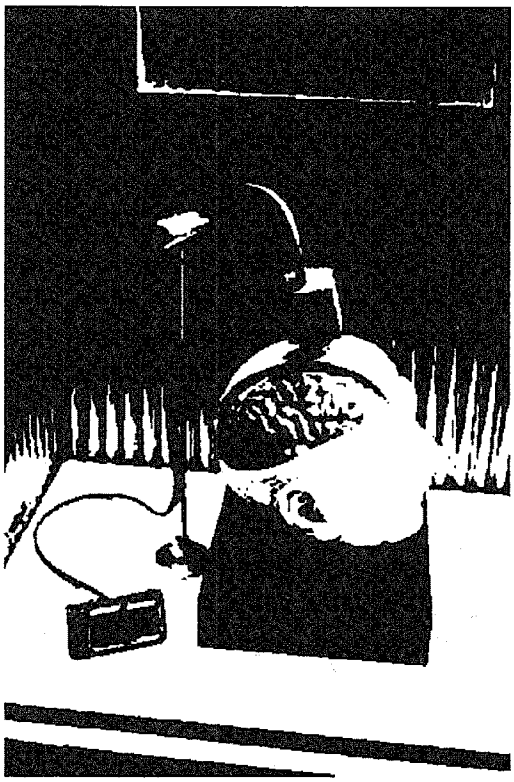


Figure 19. A Neuroprosthetic Interface. Adapted to the needs of a quadriplegic for control of his environments (Duke University)

The design and development of neural interface electrodes to the brain or peripheral nerve has been going on for more than twenty years. Figure 19 shows a mockup of a neuroprosthetic electrode system for the human brain that is meant to tap into brain signals and then through the use of a computer allow a quadriplegic to control his environment.

BIOELECTRODES AND BIOMEMS

Electrodes that interface to the brain or nervous system are the most important components of neuroprosthetic systems. There are basically of two classes of electrodes. One class does recording of bioelectric events and the other provides current to stimulate bioelectrical responses.

Both classes of electrodes need to be designed to be very robust for long term survival in the hostile environment of the human body. For this reason only the noble metals of platinum, iridium, gold, and to some extent the resistant metal tungsten are used for electrodes. Nearly all other metals would corrode in the warm saline environment of living tissues. Electronic exchange reactions occur at the electrode surfaces in both recording and stimulation modes.

Many bioelectrodes are nothing more than fine wires insulated down their length and then exposed at a needle tip where an electrical contact exists to tissue. These are usually used in research for trying to understand brain function. Sometimes a single electrode performs both recording as well as stimulation but more frequently the electrode size and material is optimized to do one function or the other.

Electrode arrays are sometimes used, such as in the case of deep brain stimulation for late-stage Parkinson's disease, which is characterized by tremors of hands. There is a "brain pacemaker" that sends electrical impulses to specific parts of the patient's brain via permanently inserted electrodes. This can stop the tremors for reasons that are not exactly known and for which the best location in a given patient to place the electrode is also not known beforehand. Thus introducing multiple electrodes and stimulating each one in-turn until the best result is found allows a greater degree of possible effectiveness and therapeutic result. MEMS techniques are being employed in this application to permit a greater number of contacts and to reduce the size of the lead wire on the electrodes.

MEMS multichannel electrode stimulating and recording electrode systems are also used in brain interface applications to record or stimulate the activity of many neural circuits. Such electrodes allow ability to record or stimulate complex muscle movements associated with the limb such as in walking or in grasping objects with the hands. Figure 20 shows an electrode system looking much like a bed of nails.

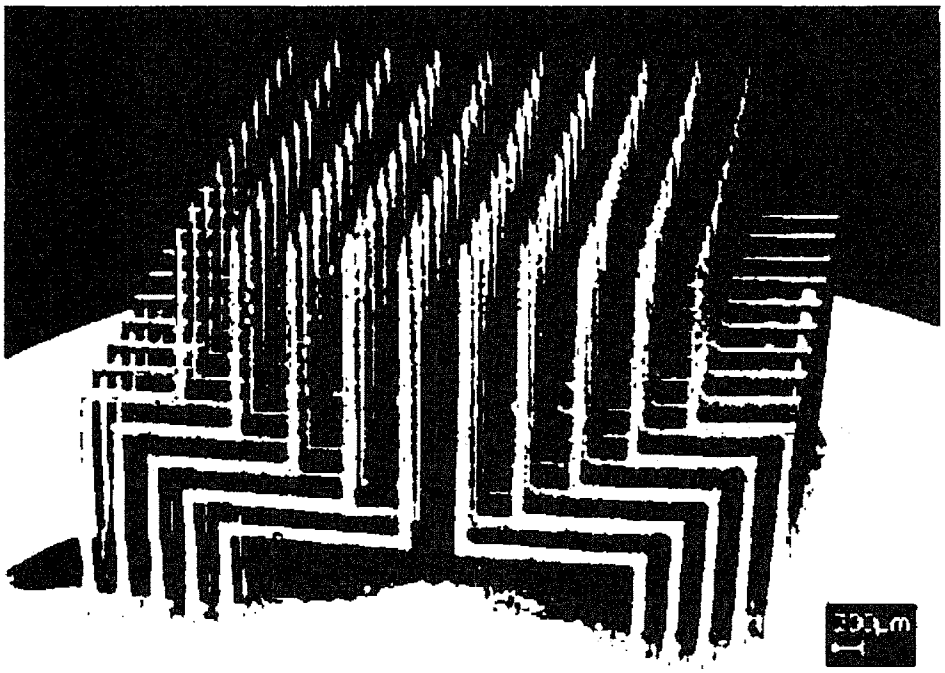


Figure 20. Scanning Electron Micrograph of a Brain Electrode Array Manufactured From Titanium and Produced by a Process of Electrodischarge Machining ("Electrical Discharge Machining and Chemical Etching", Fofonoff*, Martel, Hatsopoulos, Donoghue, Hunter)

All muscles of the body are activated by nerves that route to the brain. It is known from careful studies conducted by many investigators over the years that there is a wiring and physical mapping of the muscles to certain clusters of specialized cells in the motor cortex of the brain.

It is also known that when a person plans or strongly imagines moving part of his body, there is a complex electrical activation of the corresponding regions of the brain involving many cells over a local area.

Over the years, investigators have figured out how to interpret precursor bioelectrical events in the brain involved in planning of movements. The result is that if enough recording electrodes are placed in the brain in the motor cortex area, it is possible to determine planned muscle motion. This works even if the muscles ultimately do not move, as in the case of a quadriplegic with spinal cord injury.

Thus quite a remarkable possibility presents in that a computer program can be taught to interpret large numbers of bioelectrodes signals and so can determine a person's intent to move and then, for example, control a robotic limb that actually performs the operation.

Crude forms of these thought-controlled devices have been shown in research laboratories to be feasible, even if they are at present not practical. Among other things, the electrode interface to the brain is not yet sufficiently developed to provide large numbers of functioning electrodes that can work over prolonged periods of time.

MICROELECTRODE ARRAY FABRICATION

Neuroengineering applications in the brain can require tens to hundreds of electrodes to make contact with a similarly large numbers of cells in the brain. Large numbers of electrodes in arrays inserted into the surface of the brain cortex thus become sensitive to the timing and patterns of cell firings.

BioMEMS has been a critical part of this development. The electrode array in Figure 20 is made by a electrochemical process of using a long but stiff wire that acts like a machine tool. Electrical discharge machining (EDM) is a process that makes use of computer-aided design (CAD), that runs under computer numerical control (CNC), and that is capable of batch processing.

A block of titanium metal that will ultimately be the array is placed in an electrolyte solution and the working electrode initiates a submerged electrochemical arc. It finely removes metal by a chemical process that does not involve a lot of heat. It can generate intricate features with high aspect ratios and is capable of machining a large variety of conductive materials.

In this case, the electrode shaft thicknesses are on the order of 50-100 microns and the entire device is less than a centimeter square with 96 electrode channels. When this device is encapsulated in a polymer at its base and pressed into the brain cortex, each electrode then records from a specific set of neurons that are local to its placement.

BRAIN-MACHINE INTERFACES

A specific development in MEMS based neuroengineering has been the brain-machine interface (BMI). These devices use computers to interpret brain signals from implanted neural arrays and then use the information to control machines and setup an automated environment.

The development of such devices can have a profound impact on the quality of life for those individuals practically isolated because of their disabilities. Connected machines will enable them to enjoy the everyday things we take for granted.

For example, a visual prosthesis could potentially restore partial vision to a blind patient by stimulating neurons in the visual cortex using an input BMI. Signals could be recorded from the motor cortex using an output BMI in order to bypass a neural injury and restore some movement to a paralyzed patient. Even a simpler device that would allow a patient to move a cursor on a screen would make a significant impact.

It is generally recognized that clinical applications of such BMIs may require the activities of hundreds or thousands of neurons to be simultaneously sampled. Figure 21 is an SEM photograph of a dense array of electrodes directed towards BMI applications.

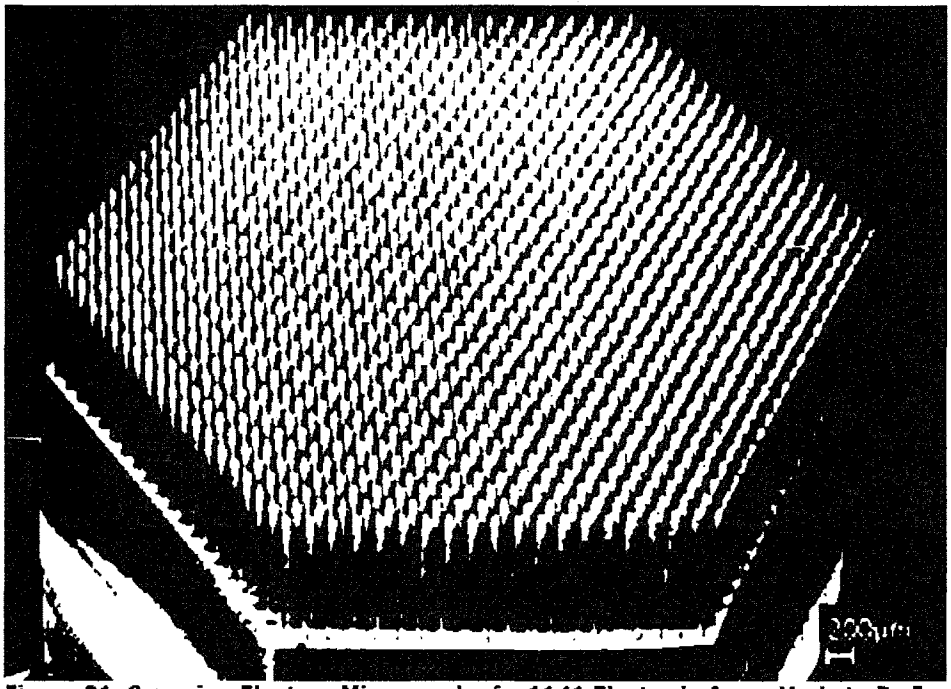


Figure 21. Scanning Electron Micrograph of a 1141 Electrode Array Made to Be Inserted Into the Surface of the Human Brain (University of Utah)

A significant problem with these devices is that when they get too densely disposed, blood flow and disruption of the natural wiring of the brain causes decline of tissue function.

Another very important issue is the biocompatibility of the material that the implants are coated with. The more biocompatible these materials are the less tissue reaction they will cause thus resulting less implant risk and longer implant period.

POLYMER BIOMEMS ELECTRODES

Polymer materials like polyimide are widely used for neuroprostheses because they are inert, flexible and metal films adhere well to them.

Manufacturing of neural electrodes can be achieved by metal vacuum deposition through photolithographic masks and by a process of RF ablation or etching. Figure 22 shows some products of NeuroNexus Inc that result from this process. These electrodes are batch fabricated and have a high reproducibility of geometrical shape, electrical properties, and mechanical properties.

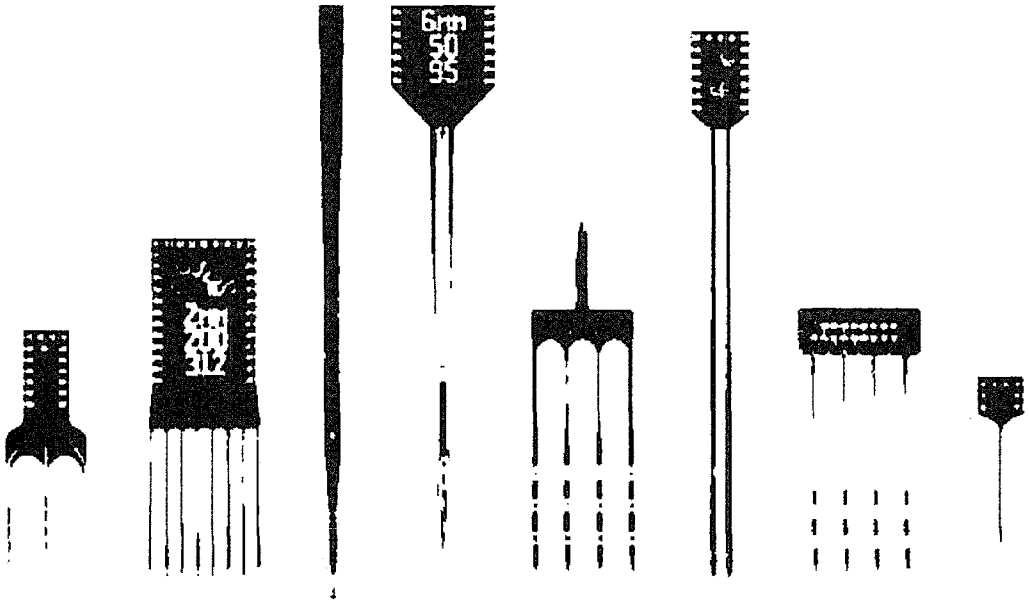


Figure 22. Polymer-Based Cortical Penetrating Neuroelectrodes Made by Processes of Thin Film Deposition and RF Etching (Neuronexus Inc)

They also have the advantage of small size, resulting in minimal displacement of the neural tissue and their long leg lengths give a high spatial resolution at various depths up to 1 cm. The multiple parallel shanks, provide horizontal spatial sampling and independent recording/stimulation among sites.

Figure 23 shows one of these devices introduced into the cortex of an exposed rat brain.

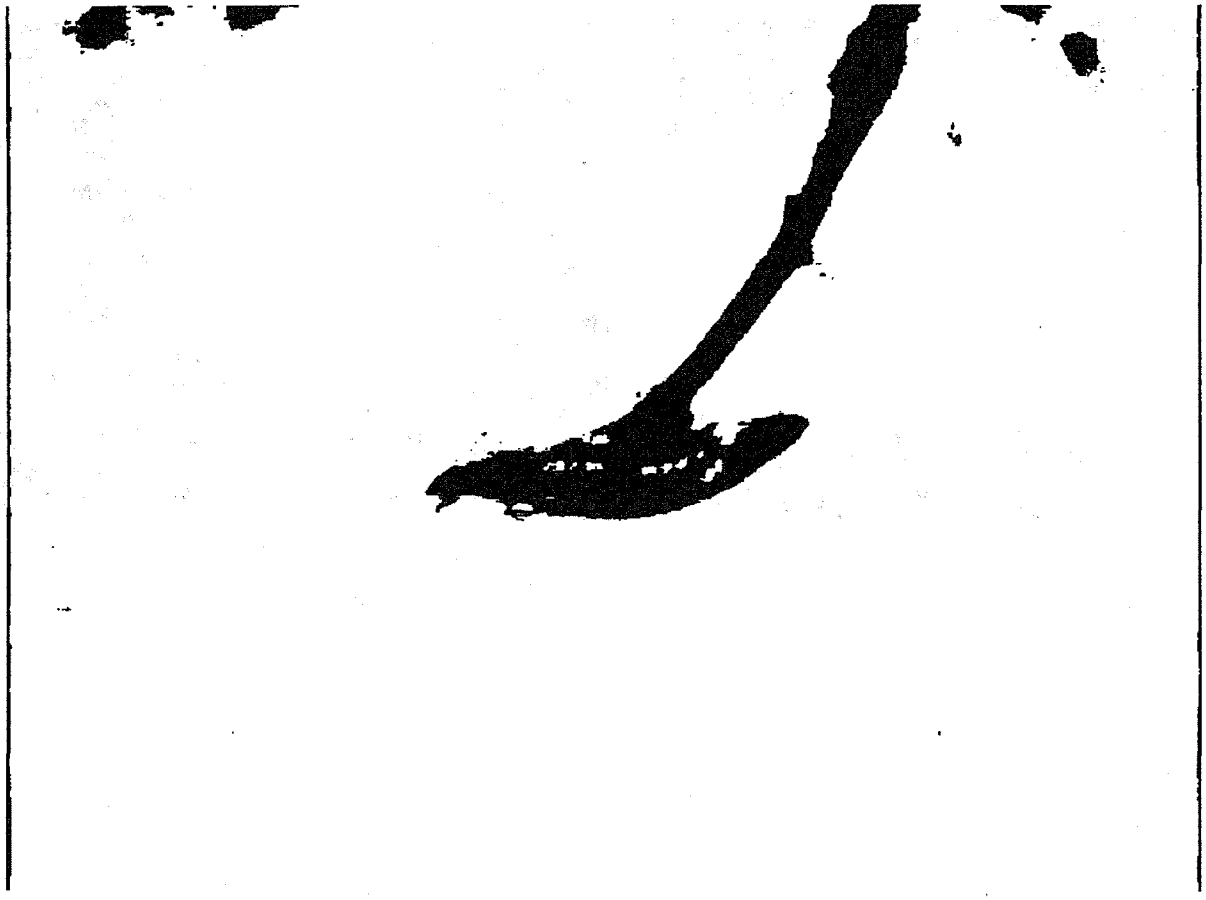


Figure 23. A MEMS Microelectrode Array Implanted Into the Cortex of a Rat Brain. The lead wires from the device can be seen. (NeuroNexus Inc.)

RETINAL DEVICES USING BIOMEMS

An example of MEMS being used in conjunction with wireless implantable systems is the retinal implant.

These devices attempt to augment vision where it has failed due to problems such as macular degeneration and retinitis pigmentosa. The basic idea is to use a miniature video camera and connect it to an electrode array in the eye such that the scene in view is converted to electrical impulses used to stimulate the sensation of sight by direct stimulation of the receptors in the eye. Figure 24 shows this idea.

Figure 25 shows a microelectrode array that has been manufactured on a polymer substrate such that it is exceedingly thin and flexible. The flexibility is needed so that it conforms to the curved shape of the back of the eye. The electrode pads seen are attached by a flexible cable.

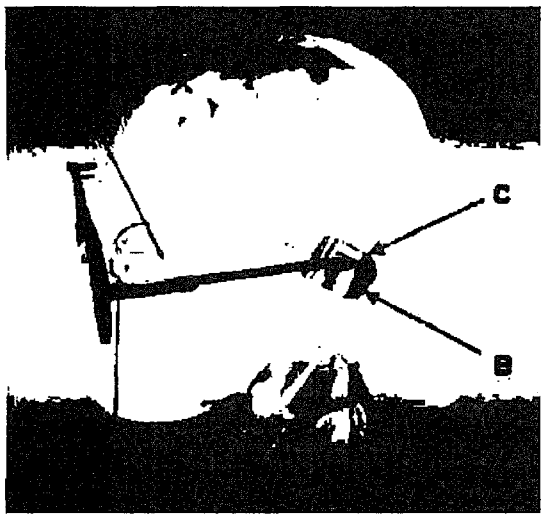


Figure 24. A Representation of the Retinal Prosthesis: (A) camera in the glass frame, (B) wireless transmitter, (C) extraocular electronic case (receiver), and (D) intraocular implant electrode array (http://biomed.brown.edu/Courses/BI108/2006-108websites/group03retinalimplants/interoccular.htm)

Figure 25. A BioMEMS-Fabricated 4x4 Microelectrode Retinal Array (http://biomed.brown.edu/Courses/BI108/2006-108websites/ group03retinalimplants/interoccular.htm)

A retinal based neuroprosthesis is designed to use electrical pulse stimulation of the remaining viable retinal cells to evoke the sensation of light at discrete points within the visual field. These apparent points of light are called phosphenes.

The strategy is to pulse the retina with sufficiently close-spaced electrodes such that the phosphenes would combine to present an organized pattern to the patient's perceptions. A completely implanted device is desired that has the electrode array connected to a pulse driver circuit, power, and signal processing. All of these components must be miniaturized as much as possible so the entire device can be implanted.

Microimplantable devices based on BioMEMS structures are being developed that capture their power from outside the body by wireless techniques. Bidirectional communication is often needed to provide feedback on device function. Some communication links need a wide bandwidth for real-time data transmission and this is challenging since such types of communication are inherently of a high power drain. Figure 26 is an artist's conception of the retinal implant designed by the Boston group.

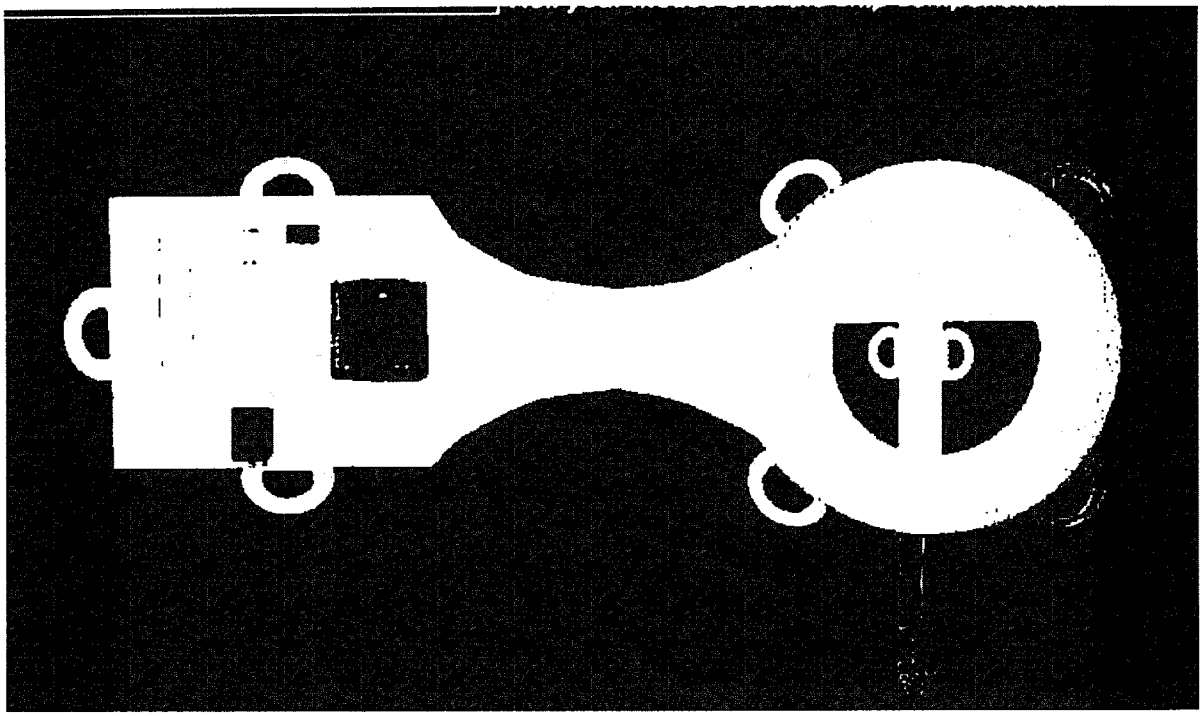


Figure 26. An Illustration of the Retinal Neuroprosthesis Created by the Boston Implant Project (http://www.bostonretinalimplant.org/implant.php?fontsize=normal&hicontrast)

The signal processing circuits are on the left while the power induction coil is the circular structure. The MEMS stimulating microelectrode array projects out towards the bottom of the picture.

The minimal size of the implant means batteries are impractical and forms of wireless power by magnetic induction, ultrasound, or solar energy are required. In addition the tissue surrounding the implant is usually very sensitive to temperature rise so the implant must have very low power consumption to ensure it will not harm the tissue.

Power by magnetic induction is widely used because it allows relatively larger amounts of power transfer compared to ultrasound and solar energy techniques. Parallel coils of wire, one inside the body and one outside, exchange energy by magnetic field coupling in accordance with Faraday's law of induction. Figure 27 is an artist's conception of the magnetic induction method of power transmission. There are two coaxial coils that couple magnetically, one on the inside of the body and one external. The energy of the magnetic field is shown as the curved lines passing through the tissue.

A disadvantage of this method is that the coils are relatively large compared to the size of micro-implants and so tends to define the minimum size. The coil size depends on the amount of energy that needs to be induced and on the implant depth with larger diameter coils being required.

This type of technology is giving way to improved methods of energy transmission into tissue for implant power by using microscale devices that, although not strictly MEMS, share many of their characteristics.

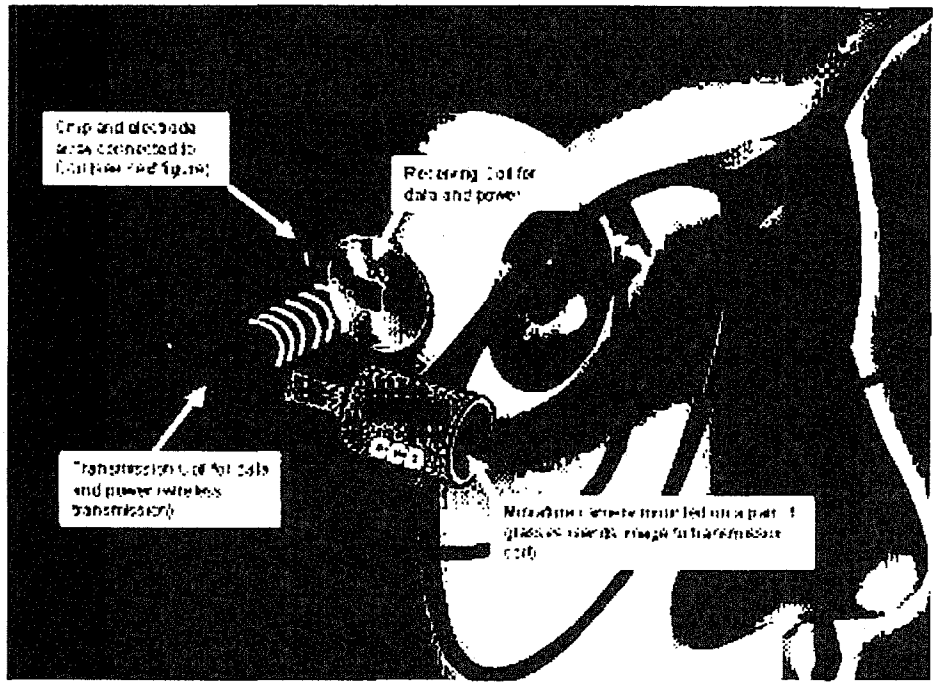


Figure 27. Power Transfer by Magnetic Induction to an Implanted BioMEMS Retinal Prosthesis. This is the Boston Retinal Implant Project.
(http://www.bostonretinalimplant.org/retinalapproach.php?fontsize=normal&hicontrast)

MICROSCALE IN THERAPEUTIC NEUROSTIMULATION

Some of the biggest excitement in medical neuroprosthetics has been in the notion that we can use micro-implantable devices rather than drugs to reduce or better control the effects of stroke, heart disease, epilepsy, and other disorders of the human condition. Bioelectrical stimulation has effects which are very specific on local tissues rather than indiscriminately affecting all tissues and so may offer fewer side effects of drugs. Such devices can be finely controlled in their effects and provide a greater flexibility in terms of treatment.

Any implanted device has to be small in order to be minimally invasive, especially in the brain. Powering of implanted devices by batteries is only practical in a few biomedical situations. Also implants may need to communicate with the outside world wirelessly. Having wires penetrating the skin is uncomfortable and could lead to infection in the tissue.

The notion of placing bioelectronic stimulation devices inside of the body for therapeutic reasons goes back to the early invention of the pacemaker. These kind of devices have internal batteries to support an electrical pulse generator that paces the rhythm of the heart.

Because the amount of energy in each pulse is relatively small the power drain is low and the pacemaker batteries can last years. The difficulty is that they are bulky, must be eventually replaced on the order of seven years, and their placement is invasive.

Variations of pacemaker devices are used in brain neurostimulation for treatment of Parkinson's disease characterized by the involuntary tremors of the hands and body. In this case the battery pack must be placed outside the brain, typically in the upper chest and a long catheter wire tunneled through tissues to the specific parts of the brain to be treated.

Research at Arizona State University has been directed towards overcoming the problems of bulk and need for battery replacement through an approach where the implanted neurostimulation devices are made exceptionally small and where they derive their power by a process of induction from the outside of the body. Figure 28 shows one of these devices. The tiny size of the device reduces tissue trauma upon insertion. Small electrodes at either end of the device contact neural tissue and apply electrical stimulating pulses.

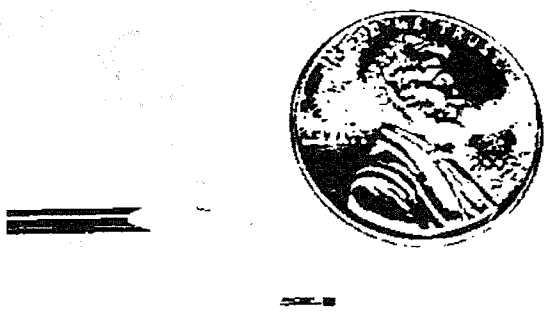


Figure 28. A New Generation of Implantable Neurostimulation Devices Can Pass Through the Lumen of a Syringe Needle. This device was made at ASU and is powered by ultrasound energy. (Towe et al.)

The patient wears a type of powering-patch on his body over the micro-implant and is supplied by a small cell-phone like device having batteries that provides the energy for driving the patch.

A coin-sized transducer on the skin directs ultrasound energy at about 1 MHz frequency towards an implanted microdevice.

A type of piezoelectric plastic material known as PVDF is configured as a ultrasound receiver and works to change pulses of the sound wave energy into a rectified electrical current. This current is then used to stimulate tissues.

Due to the fact that ultrasound is a mechanical vibration and carries significant energy in a vibrating wave, the energy transfer across the skin can be more easily achieved than magnetic induction to a similar depth and size and so provide the needed currents for neurostimulation.

Figure 29 shows a bioelectrical stimulator configuration. The implanted device is 0.9 mm x 1.2 mm and contains a Schottky diode. The piezoelectric output current response of PVDF to the ultrasound is increased by stacking thin 25 micron sheets of the material connected so they are in electrical parallel. With bonding layer thicknesses in-between, the overall thickness of the stack is on the order of 250-350 microns and forms a solid structure.

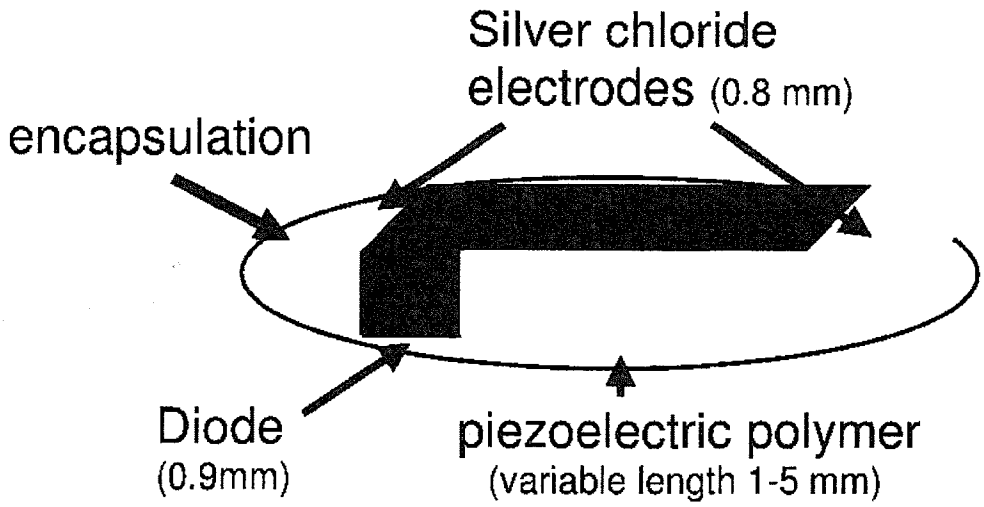


Figure 29. Illustration of the Internal Construction of the Ultrasound Powered Neurostimulator Developed at ASU. Platinum ball electrodes at either end are the contacts to the tissue. (Towe et al.)

This device has been implanted in rats and shown effective in neurostimulation under a wide variety of conditions. It appears suited to near-surface neurostimulations for relief of pain, and in the potential treatment of a variety of nervous system disorders.

MEMS in Microfluidics

Another major area of application of BioMEMS is the control of fluids on very small scales and quantities. This capability is important in rapidly testing blood chemistry from single drops of whole blood, in the laboratory for clinical chemistry, and in working with very small amounts of DNA derived from cellular extracts.

Manufacturing small channels that conduct fluids is relatively straight forward using photolithography. A computer generated optical mask is used to expose patterns onto photoresists, then a process of etching by plasma or chemicals is used to carve out parts of the substrate.

Substrates are often glass since it is cheap, easily formed, and is a reasonably inert and biocompatible surface. Figure 30 shows some of these kinds of configurations. Glass is easily etched by hydrofluoric acid, but also forms of sandblasting with a fine grit have recently been developed to cut holes in glass.

Finely carved capillaries in glass can allow nanoliters or even picoliters of fluid to

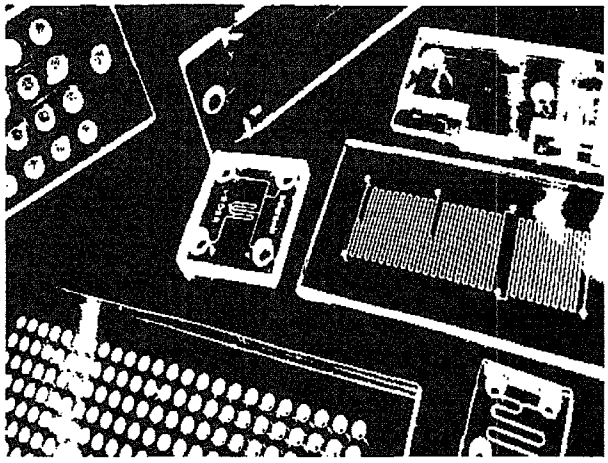


Figure 30. Microfluidics in Glass. Microfluidics in glass take advantage of MEMS to produce microscale devices that can do complex chemical analysis. (http://www.i-micronews.com/interviews/Micronit-Microfluidics-discusses-product-diversification.html)

move through the channels where processes of separation, filtering, mixing, heating, reaction, and waste disposal can occur in a programmed sequence. Color changes after a sequence of chemical steps might be used to indicate the presence and concentration of an analyte. Optical absorbance or transparency can be read by a simple light emitting diode (LED) and photodetector system that measures light passing through the glass sample cell.

LAB CHIPS

A class of microfluidic devices known as lab-chips or Lab-on-a-Chip (LOC) have made their appearance in the market place. These devices are miniature chemical analysis systems that replace many of the manual and laborious steps associated with analytical chemistry. They are generally produced by MEMS techniques and often, but not always, incorporate electrical systems on the devices.

Automated MEMS-based lab-on-a-chip platforms have become an established system for basic life science research and drug discovery. They are used to assess the characteristics of isolated DNA or proteins.

One of the most useful products of bioMEMS instrumentation is the ability to manipulate small quantities of DNA using microfluidic pathways to perform chemistry with only the content from a single cell. BioMEMs enable new techniques in genomics (the study of sets of genes, gene products, and their interactions) and proteomics (the study of proteins, the expression of genes in health and disease).

These systems are being made now by companies including Affymetix, Caliper, Nanogen, and Agilent for processing samples on a microfluidic chip for separation and detection. Figure 31 shows the variety of such systems currently on the market.

Such devices can replace a number of gel electrophoresis operations and substantially improve work flow sample handling and analysis, lower per sample analysis by 10X and minimize sample and reagent use.

The physics of these microfluidic systems enable some unique features, including smooth laminar flow through the microchannels, high surface area to volume ratios, small thermal mass, and strong effects by electric fields. Microfluidic devices also lend themselves to enhancements for single cell detection, fluorescence detection, sorting schemes, and unique fluid separation methods.

The advantages of lab-on-chip devices include:

- Improved fluid transport by electrokinetic effects and miniaturized pumps.
- Efficient molecular and particle separation and immobilization.
- Smaller sample requirements and carrier volumes.
- Reduced reagent consumption and expense.
- Integration of channels, mixers, separators, reaction chambers, electrodes, and detectors into single devices.

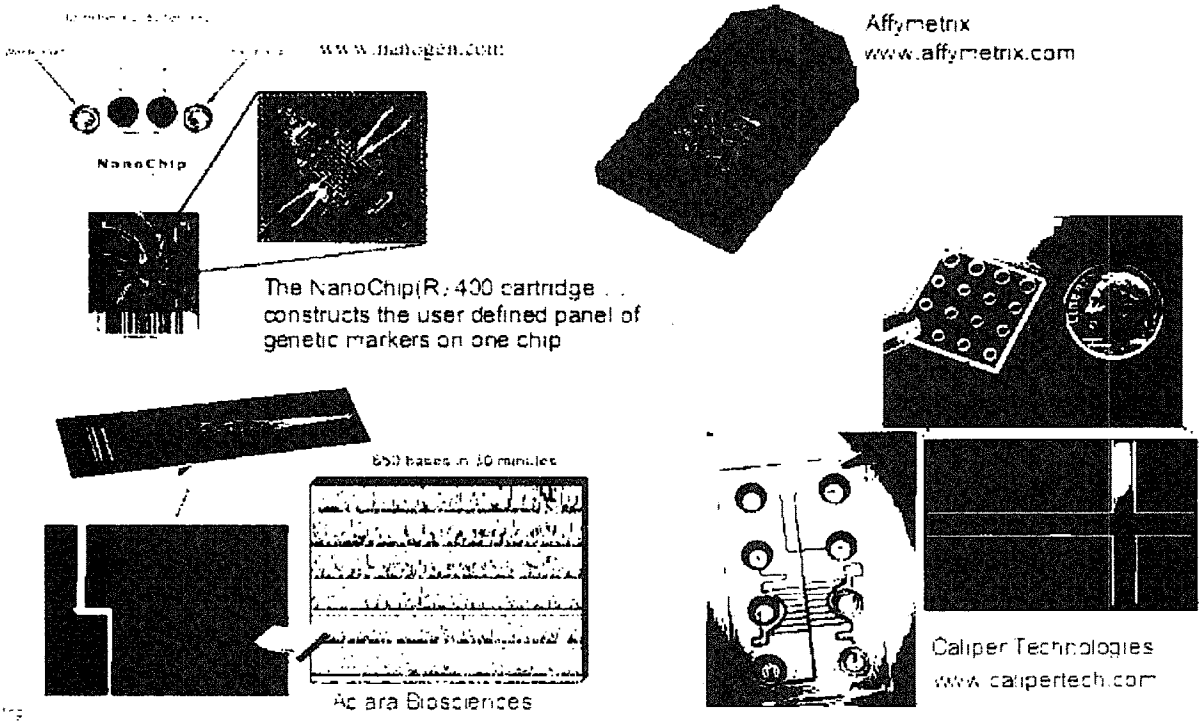


Figure 31. Commercial Lab-Chip Devices That Allow Analysis for Biomarkers of Various Diseases Using on-Chip Electrophoresis in Microscale (collage from manufacturers listed)

These MEMS devices incorporate micro-scale fluidic components, sensors, actuators, and customized surfaces created by chemical modification or coating with inorganic and organic materials; enabling tissue engineering and cell patterning studies along with the microfluidic control of the culture environment.

NASA SPACE APPLICATIONS FOR MICROFLUIDIC SYSTEMS

NASA has supported at Arizona State University, the development of new types of microfluidic systems directed towards growing cell cultures on a chip. The specific application is detecting the effects of space flight and microgravity on living things at the cellular scale.

Space presents a number of stresses on the cells of the astronaut's body from electromagnetic fields, microgravity, and space cabin environments, which in combination have effects that are likely different than any single stress. Beyond affecting the astronaut directly, space flight can change any organism, and actually makes some bacteria more dangerous.

Cells can be grown to represent the cells of the astronaut's body. Changes in DNA expression or molecular stress markers in cultured cells can be used to infer effects of space.

Culturing mammalian cells in the laboratory generally requires constant attendance and adjustment, but astronauts have little time for routine culture procedures. Thus NASA supported the design of an automated microfluidic system to perform all the steps

needed to culture living cells, in volumes of less than a milliliter. The device contains thermopneumatic micropumps, microvalves, culture media reservoirs, cell culture chamber, and optical readout systems to detect fluorescent markers that the cells express when subjected to certain types of stress.

Sensors for glucose, oxygen, and pH monitor the culture medium and work with a servo system to keep culture conditions within a narrow range. One of the designed cell culture systems can be seen in Figure 32. The system is made from micromachined acrylic, and has fluidic pathways defined as channels in the plastic. The entire system is first laid out using a CAD (computer aided design) system and then machined to fine tolerances and chemically polished.

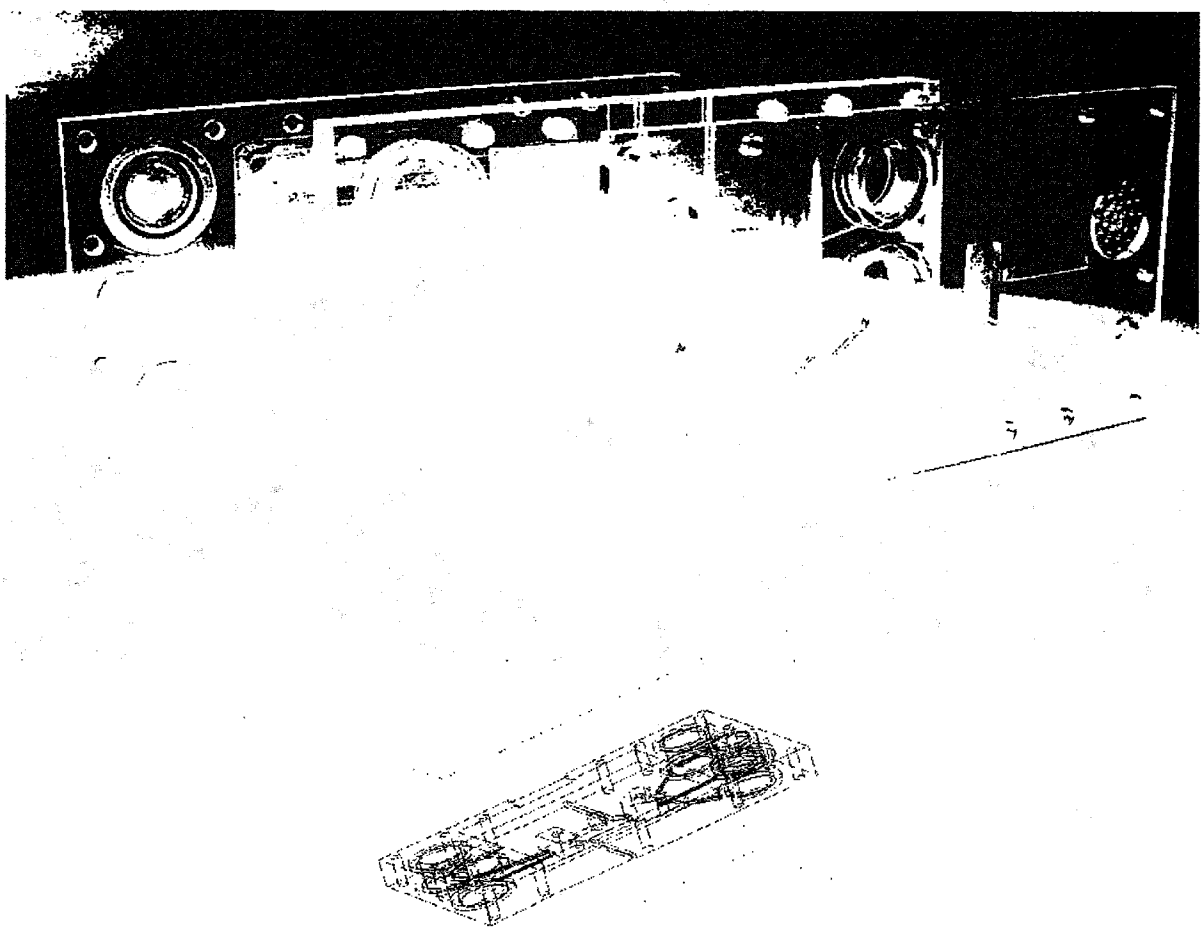


Figure 32. Design of an Automated Cell Culture System for NASA Space Applications Incorporating Microfluidics and Integrated Optical Components (Towe et. al.)

Figure 33 shows a more compact version of the device which contains two integrated optical cells designed to monitor the expression of green fluorescent protein (GFP) and

red fluorescent protein (RFP) in E. Coli bacterial cells genetically engineered by ASU microbiologist Dr. Valerie Stout. These bacteria glow when the *rec-A* gene is active, indicating a certain type of stress or damage to the bacteria due to the space environment.

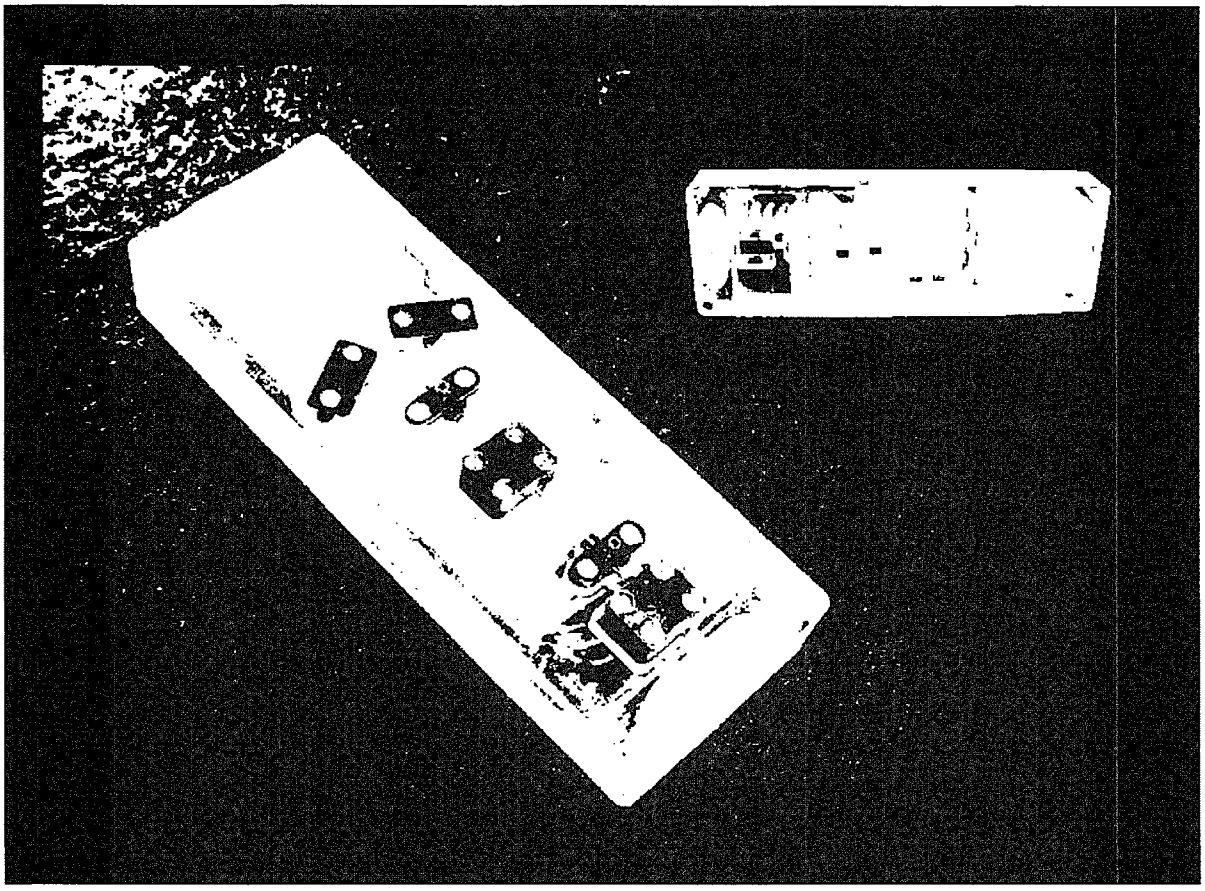


Figure 33. NASA Space Application of Microfluidic Cell Culture System Made by Micromachining (Towe - ASU research)

Microcantilever MEMs Sensors

The microcantilever is one of the simplest MEMS based biosensor devices, with wide applications in detection of very small amounts of specific chemicals through a process known as affinity sensing. This can detect gas composition, antigens in the air, toxic gases and particulates.

The use of microcantilevers as transducers for chemical and biochemical sensing has only become widespread in the last few years, mainly due to their use in the development of microsensor arrays. The idea is quite simple: the surface of the cantilever, a thin rectangular beam clamped at one end, is chemically modified to react with specific compounds. The mass deposition that follows the chemical reaction causes a stress, and this leads to a detectable bending of the cantilever as illustrated in Figure 34.

Microcantilever based sensors fall into two categories: static sensors and vibrational sensors. Static sensing microcantilevers are usually covered with a gold film, which is then coated with a substance having a specific affinity. Upon binding the specific analyte (such as a protein), stress is generated resulting in bending of the microcantilever.

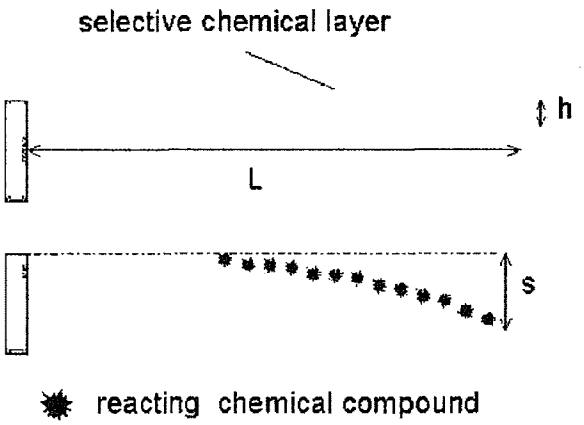


Figure 34. Principle of a Microcantilever That Bends When It is Loaded With an Adherent Mass

The deflection is often measured using a narrow light beam from a small laser. Light reflected off the bottom of the cantilever falls onto a surface, where a position sensitive detector (PSD) can determine how much the beam bends. Typically the bend is exceedingly slight, but the sensitivity of the optical readout system is high enough that small amounts (nanograms to picograms) of adsorbed material can be detected. These devices are mostly used for measuring things in the air and not well suited for immersion in fluids.

In vibrational sensing, an external motor is used to vibrate the microcantilever through a certain range of speeds, and a sensing mechanism then reads out the amplitude of the vibration. By seeing at what speed the cantilever vibrates best, the resonant frequency is obtained. As bioparticles bind to the microcantilever, the resonant frequency will be slower due to the increased mass.

The mass of attached analyte can be determined from the frequency of the microcantilever. The microcantilever has its own natural frequency ω. When a biomolecule binds to the microcantilever, it changes the mass of the microcantilever. This in turn affects the resonant frequency. We can use that change to determine the mass that has been attached to the microcantilever.

In some sense it is like a tuning fork that changes its pitch when touched. The surface has an affinity for adhesion of a certain material and nothing else. When these materials are present, they adhere and increase the mass and so the pitch moves lower.

The sensitivity of these systems to the loaded mass increases as the mass of the cantilever beam decreases and with it the resonant frequency increases. Decreasing the overall dimensions of the beam results in an corresponding increase in their sensitivity.

Thus the trend has been to employ MEMS technology to reduce the size of the cantilever beams to microscopic proportions. In such cases, even the mass of cells or virus particles can be measured. Figure 35 shows a microcantilever system that is on the order of one micron in width and 10 microns in length. This cantilever beam was used as a mass detector, with sensitivity to a single vaccinia virus particle (vaccinia virus forms the basis of the smallpox vaccine).

Biomolecular sensors with the ability to "multiplex," or to detect a large number of different molecular species at the same time, are being developed for cancer diagnostics and therapy monitoring.

Figure 35. Scanning Electron Micrograph Showing a Cantilever Beam With a Single Vaccinia Virus Particle

The secret to good performance is finding a coating that has a high sensitivity for a specific material of interest. Antibodies are often used as coatings since they can be tailored to bind to many different molecules. Even so, there are often problems of antibody shelf-life and they are not perfectly specific and so experience interference from other chemicals in the test sample.

Figure 36 illustrates the affinity coating. Antibodies for different proteins are coated onto different micro-cantilever beams, making each beam a sensor.

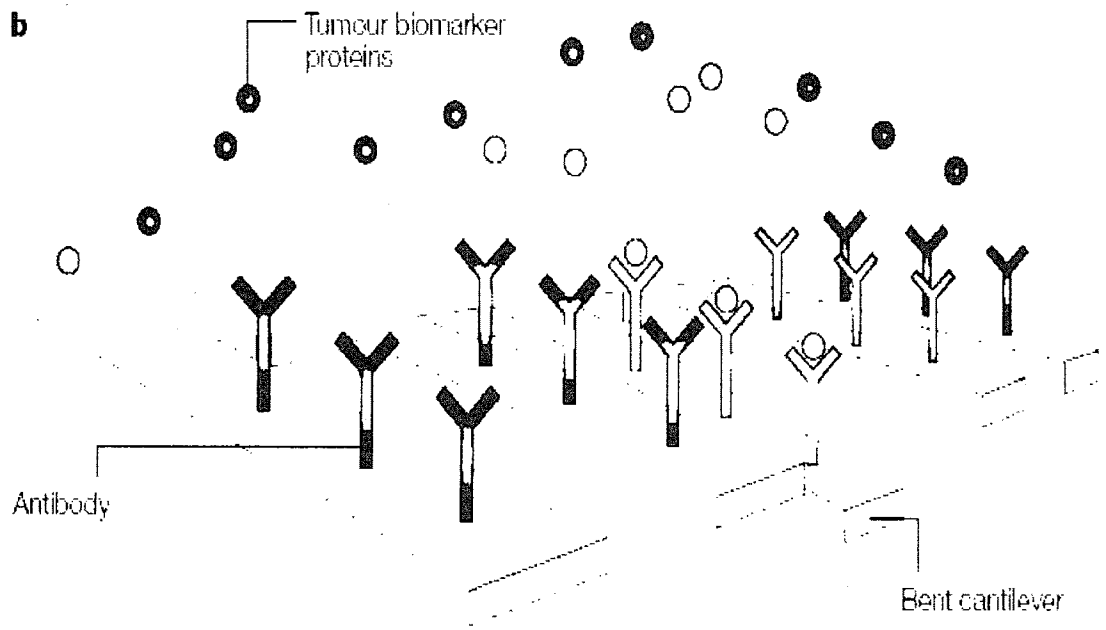


Figure 36. Antibodies Attached to a Cantilever Array Create Different Specificities to Substances in Blood That are Indicative of Cancer

Conclusion

MEMs are characterized by small dimensions, enabling new applications of physical principles to make sensors and actuators of superior characteristics.

Physical properties like surface tension and electrostatic charge dominate the operation of the devices, and new fabrication approaches are required to make them. The economic driving forces for this miniaturization are strong for health care and biology.

Advanced fabrication processes are still being discovered to further miniaturize electromechanical systems and to bring us into the realm of nanomechanical systems (NEMS). The new class of NEMS devices are expected to provide a higher performance in applications such as sensors, medical diagnostics and in the electronics industry. Some devices will enable experiments on the structure and function of individual biomolecules.

This field is expanding rapidly. Searches of the scientific literature show new ideas being developed in a number of laboratories throughout the world.