A disposible biochip for low-cost DNA analysis

Largely driven by the human genome project, the application of micro-systems technology to the field of molecular detection has resulted in a variety of devices, such as DNA-arrays and biochips. Presently, these devices are extensively used in genetic research, research into diseases and drug discovery. These biochips are typically 'high-end', based on silicon technology and contain between 10.000 and 100.000 discrete sites. At each site a small quantity of synthetic DNA – the probe DNA, some 10's to 100's of nucleotides long – is immobilised. Each site carries probe DNA with a different base-pair sequence – the recognition sequence.

The DNA to be investigated, for example from a patient, is prepared by extracting the relevant short pieces and linking them with a fluorescent dye or other marker. This target DNA, in solution, is then flushed over the chip. The target DNA will bind to the probe DNA at those sites where the target base-pair sequence matches the recognition sequence. The bound complex is then detected by optical fluorescense of the marker.

The Market

The world market for biochips is estimated at 500M€ in 2000, at a compound annual growth rate of about 65% with medical applications representing the large majority of this.

The current generation of biochips is too expensive for routine diagnosis or screening of patients. A new market for cheaper, 'low end' biochips for mass use is now emerging. These chips would contain typically some hundreds of sites with probe DNA sequences. They would be used for diagnosis of:

- infectious diseases: bacterial or viral, such as HIV or hepatitis.
- transplant tissue compatibility
- genetic pre-disposition to conditions like cancer, neuro-degenerative disorders, auto-immune diseases, etc.

Presently, this type of diagnosis is performed in the clinical laboratory, using an array of specialised techniques, including cell cultures, and molecular biology-based techniques. These are time-consuming, not automated, and do not provide all relevant clinical information. Other disadvantages are the handling of substantial amounts of chemicals, and the risk of contamination.

The introduction of biochip systems will reduce consumption of chemicals, avoid contamination, and improve throughput and automation in the clinical laboratory. A longer-term objective is the development of point-of-care systems, aimed at obtaining a fast diagnosis "at the bed side".

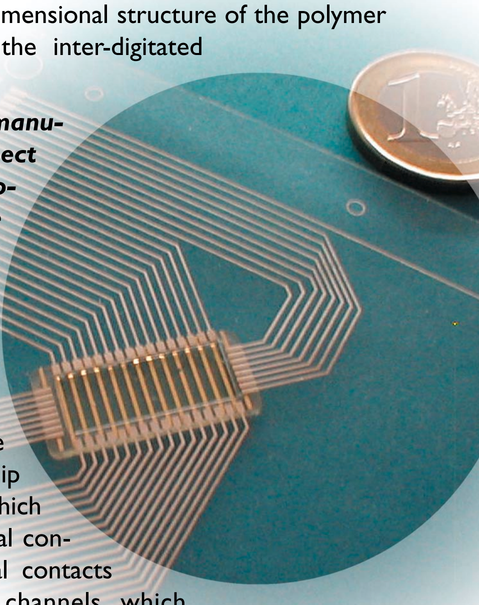
Innovations

The project has developed core technologies in the mass-manufacture of low-cost biochips. The major areas of innovation are:

- ***The detection of the binding of target DNA to probe DNA is made electronically.***
  In order to facilitate a fast chip read-out – and to avoid costly fluorescence detection equipment – the detection of binding of DNA to probe DNA is made electronically. Each discrete DNA probe site – called a sensel - consists of inter-digitated gold electrodes connected to electrical contacts at the edge of the chip. The present chip contains 128 sensels, measuring 200 by 200 micro-meter each.

- ***A manufacturing process for the polymer chip bodies with sub-micron features, using micro-injection moulding, was pioneered.***
  The process is based on the initial manufacture by lithography of a master-mould in silicon. From this, metal mould inserts for the micro-injection moulding process are obtained. Because the moulding process must accurately reproduce three-dimension at sub-micron structures, it is a highly demanding process.

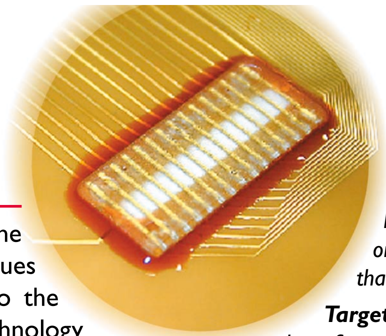
- ***A novel technique for the deposition of the inter-digitated gold electrodes was developed.***
  This technique uses oblique evaporation of gold, utilising the 'shadows' of the three-dimensional structure of the polymer chip bodies to create the inter-digitated electrodes.



- ***Novel solutions for manufacturing the interconnect structure and the micro-fluidics structure of the chip were pioneered.***
  A screen-printing process deposits insulating layers and electrically conducting elements. The process leaves only the sensel areas exposed. The chip body is then flip-chip bonded to a sheet, which carries both the electrical connections to the external contacts and the micro-fluidic channels which transport the target DNA to the sensels.

- ***DNA immobilisation and probe dispensing technology was developed.***
  The challenge is to accurately dispense a large number – 128 on this chip – of different DNA probe reagents, while avoiding cross-contamination. The quantity for each sensel is typically 10 nano-litre.

All elements are now in place to further elaborate a multi-parameter electronic assay, and the related electronic read-out system.

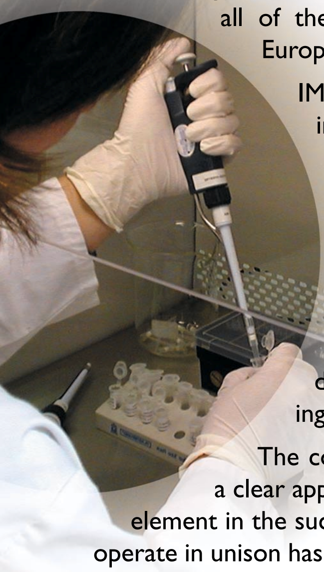


The multi-disciplinary Project Team

The development of biochips is considered part of the field of nano-biotechnology. Here, the tools and techniques of microelectronics and micro-systems are applied to the field of molecular biology or cell biology. Nano-biotechnology is an eminently multi-disciplinary field, which is well reflected in the partnership of this project. The biochip requires a wide range of technologies, which are traditionally not linked.

The primary driver and end-user of the development is the Belgian company Innogenetics. They develop and market a range of molecular diagnostics products for the analytical laboratory. This includes a DNA detection system where the probe DNA is immobilised as macroscopic lines on cellulose strips (typically probe sites of 1 by 2 mm), and where binding is detected by colorimetric detection of a precipitation reaction. The biochip developed in the project is aimed to be the next step in this technology.

In order to pursue this development, a project team was needed to give access to all the necessary competencies. As not all of these could be found at national level, a European partnership was set up.



IMEC and IMM developed the manufacturing process of the polymer chip bodies, based on IMEC's micro-systems expertise, IMM's knowledge on micro-injection moulding, and IMEC's expertise in deposition of metal layers by evaporation. Cranfield university took the responsibility for the screen printing and interconnection developments, while Biodot developed the automated reagent dispensing system.

The co-ordinatorship by Innogenetics provided a clear application orientation, which has been a key element in the success. To make this multi-disciplinary team operate in unison has required considerable investment of time and co-ordination effort. However, this has paid off through the development of an entirely novel device.

An application example

Transplanted organs run the risk of being rejected by the receiving patient's immune system. This risk is affected by the degree of similarity of a certain set of genes between the patient and the organ. The risk can be characterised by a few hundred sequences DNA base pairs. The new biochip will allow a more complete, faster and cheaper testing of organs and recipients in order to get a better match in a time-critical situation.

Glossary

***Probe DNA**: DNA strands immobilised onto the chip, containing the DNA sequences that one is looking for.*

***Target DNA**: the DNA under test, for example that of a patient.*

***Biochip**: a miniaturised system for the detection of biological entities (cells, viruses, bacteria), through the specificity of their DNA or protein.*

***Point-of-care**: the use of biochip for a faster diagnosis, executed near the patient.*

***Sensels**: the individual detector elements that make up the biochip array.*

***Recognition sequence**: the specific sequence of base pairs on the probe DNA. Each sensel carries probe DNA with a different recognition sequence. Only target DNA strands which contain a matching sequence will bind to the probe.*

An innovative production technique for affordable sub-micron electrode arrays for molecular diagnostic purposes

PolymerMicroSensorFab
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