NABIS

Project no. **NMP4-CT-2003-505311**

Project acronym: **NABIS**

Project title: **Nanobiotechnology with Self-Organising Structures**

Instrument: **STREP**

Thematic Priority: **FP6-2002-NMP-1**

Final Activity Report

Period covered: **from 1 January 2004 to 31 June 2007**
Date of preparation: **26 September 2007**

Start date of project: **1 January 2004**

Original Duration: **36 month**
Extended to **42 month**

Project coordinator name: **Prof. Johan Roeraade**
Project coordinator organisation name: **Kungliga Tekniska Högskolan**

Publishable final activity report

1. Project execution

Objectives

The NABIS research project has been a 3.5 years effort which started in January 2004, with the overall goal to study and develop novel technologies, to be used in the development of the next generation of high performance biochips. Biochips are anticipated to become of major importance in areas like accellerated drug discovery, diagnostics and personalized medicine. One of the fundamental technologies, developed in the NABIS project relies on a predictable self-organization of fluids, where both static (e.g. microarrays) and dynamic (e.g. microfluidic systems) formats are utilized. The formats can be combined to overcome current bottlenecks in the automation of high throughput assays, and will also be used for the development of methods for high throughput biochip fabrication. Other complementary technologies deal with surface-expanded high density bioprobe arrays, including the use of novel polymers. Also, novel magnetic bio nanowires, controlled by an external magnetic field onto a platform of magnetic nanodots, will be explored. It is anticipated that the use of these technologies will provide a possibility for a controlled increase of ligand density, as well as enhanced kinetics, resulting in improved sensitivity and faster assay performance. In this context, a technology based on externally applied surface acoustic waves will also be utilized, to yield an efficient agitation of nanodroplets. Finally, a nanoelectrochemical detection system will be developed. The technologies will be combined in various ways to result in optimized biochip systems, which will be tested using standard bioassays, and the outcome will be compared with performance data, obtained from similar experiments with state of the art biochips.

The *contractors involved* in this project are:

Partic. no.	**Participant name**	**Participant short name**	**Country**
1	Kungliga Tekniska Högskolan (coordinator)	KTH	Sweden
2	Institut Curie/CNRS	CURIE	France
3	Naturwissenschaftliches und Medizinisches Institut an der Universität, Tübingen	NMI	Germany
4	Ecole Normale Superieure/CNRS	ENS	France
5	DiagnoSwiss SA	DS	Switzerland
6	Advalytix AG	AVX	Germany

The work performed in this project consists of the following:

* Development of methods for controlled surface expansion in nanodomains, including the fabrication of high-density arrays with surface-enlarged reaction dots. The surfaces will be functionalised with novel, self-assembling polymers.
* Establishing a new technology for self-organisation and replication of dot arrays of defined nanovolumes of aqueous liquids (reagents or samples) onto a microchip.
* Preparation of clusters of magnetic nanoparticles, arranged into dots or wires.
* Grafting different chip surfaces with functional biomolecules is another fundamental element of the project. This includes the development of novel surface modification methods, and the development of a range of assays, suitable for implementation in the new microchips.
* Development of generic tools for preparing and manipulating self-organized nanodrops of aqueous liquids in a flow of a hydrophobic "host" fluid. The nanodrops will be utilized as microreactors and the usefulness of the principle will be demonstrated with an example of high throughput nanoliter-sized PCR amplification, combined with laser fluorescence detection.
* Design of a device, generating surface acoustic waves to be used for agitation of fluids in the miniaturized liquid domains.
* Nanoelectrochemical technology for detection in connection with the bioassays on a chip-format will be developed.
* In the final phase of the project the various technologies will be combined to obtain an optimum performance.

Final Results

Basically, all goals, which have been set in the original project plan, have been achieved.

Several methods for preparation of controlled surface- enlargement have been established. This includes e.g. the preparation of nanopillar-like structures, using both silicon dioxide and polymers as a substrate. An example is shown in fig. 1

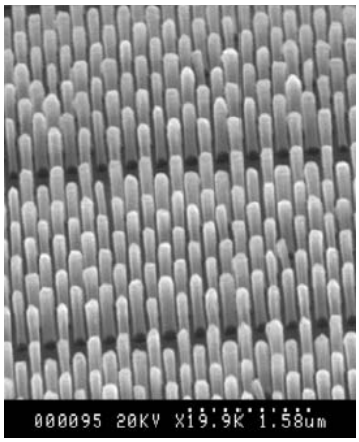

Fig 1: Ordered polymer nanopillars
Note the high resolution and high aspect ratio.

Also, we have succeeded in making magnetic nanodots, both in array formats in into channels (fig 2).

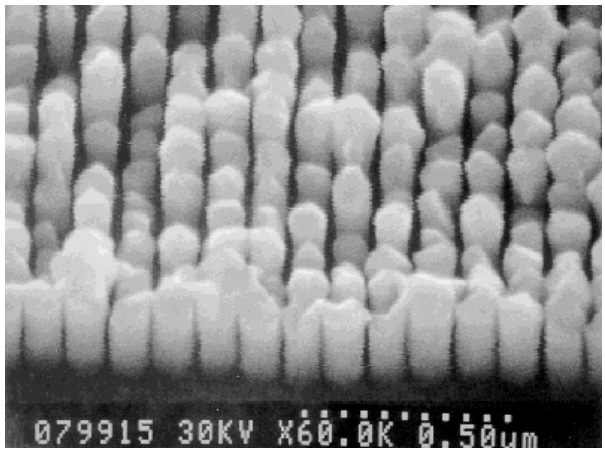

Figure 2. Scanning electron micrograph of nano-dot arrays formed by electroplating, defined by nanoimprint lithography.

The nanodots are used as anchoring points for functionalized magnetic nanoparticles, which then form nanowires. An example of long nanowires is shown in figure 3

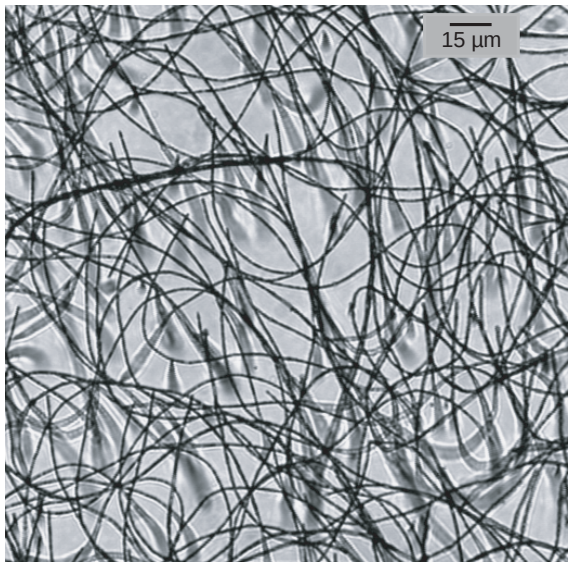

Figure 3: Photograph of long magnetic nanowires made by submitting a mixture of magnetic particles to a 25 mT magnetic field during 5 min. The chains are bent by gravity after field removal.

Several methods have been developed to couple polymeric materials to the surfaces. These polymers can serve as an intermediate surface for coupling biomolecules.

An example of such a result is shown in figure 4

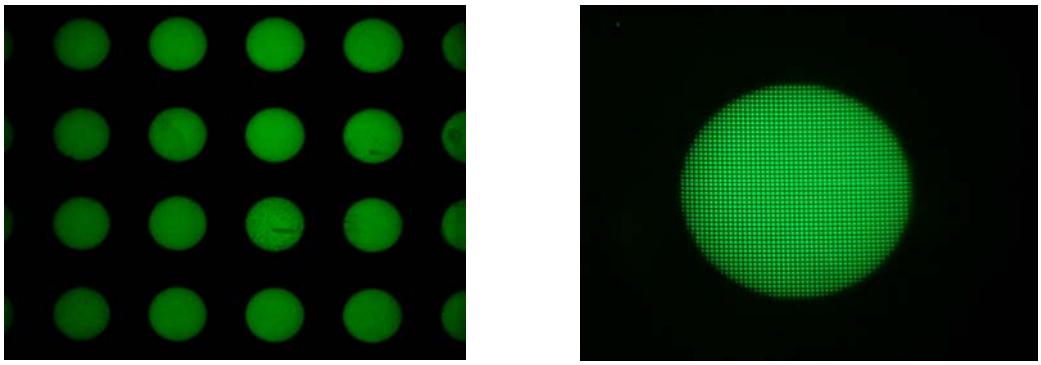

Figure 4. Fluorescence images of poly-L-lysine patterns (defined by lithography). Each area has a diameter of 180µm and fulfilled or half filled of dot arrays of 2µm diameter and 4µm period.

Several technologies for controlled and permanent surface hydrophobization have been developed, which allows a creation of confined, well defined hydrophilic areas (dots) for self-organisation of very small volumes of solutions, containing bioactive molecules. The technologies include the preparation of “super-hydrophobic” surfaces using the lotus-leaf concept (see an example in figure 5) as well as the use of elevated targets with an abrupt change in contact angle, which provides a maximum of droplet confinement and increases the robustness of sample deposition.

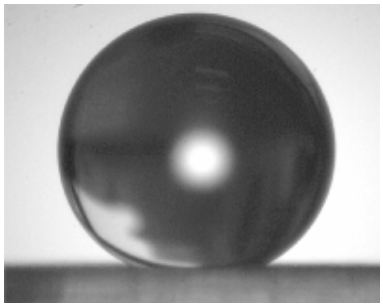

Figure 5. Microphotograph of a water droplet on patterned hydrophobic surface of a pillar array of height 10 µm and a period of 10µm, showing a contact angle larger than 150° and super hydrophobic wetting properties.

Also, new concepts for automated spotting of both micro- and nanodroplets of liquid in various array formats have been developed. Figure 6 shows a typical example of a microscopic slide, where 900 sample spots were deposited.

The software platform for this dispensing system is extremely reproducible and the system has a great flexibility in dealing with different formats.

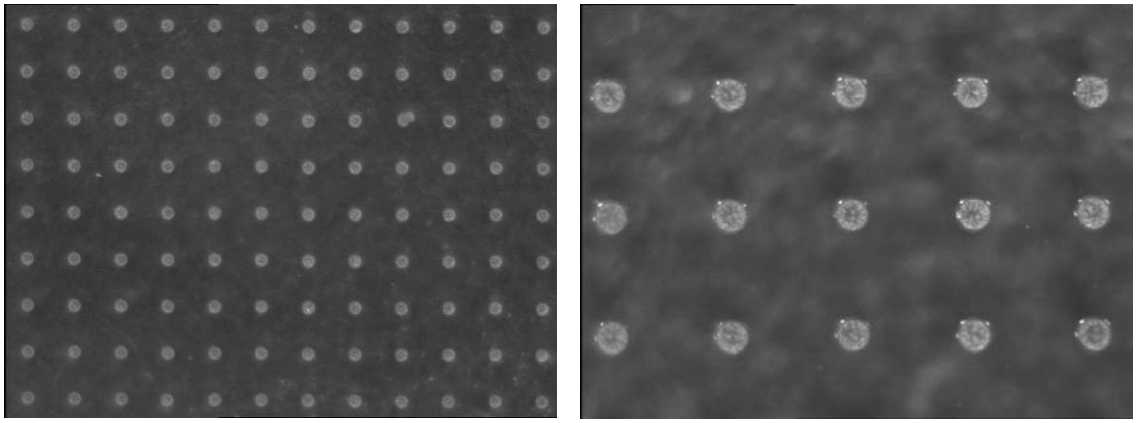

Figure 6. Left: Picture showing part of a 900 spot matrix after deposition of an anti-mouse IgG solution. Right: Magnification of part of the left picture.

We also demonstrated that the system can handle extremely “sticky” protein solutions, like concentrated solutions of fibronectin or collagen. This feature was used in subsequent cell adhesive assays. It is known that “sticky” protein solutions are very problematic for conventional instrumentation like piezo-electric devices or contact spotters.

Also, a new frontline technology was developed for handling matrices of different solutions simultaneously. The demonstrated capacity of the system was 2400 spots/min, which correspond to a nominal capacity of ca 3.000.000 spots/day. The individual sample volumes that can be handled are in the nl - pl range. It is anticipated that the technology has a considerable potential for high speed fabrication of biochips, but it should also open up new possibilities in other areas like material science, combinatorial chemistry, development and optimization of catalysts etc.

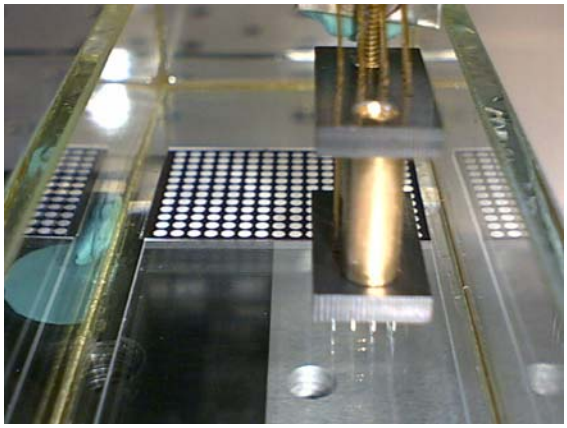

Figure 7. Photograph showing a 16-channel matrix “print head”, as well as microarray substrate and sample chambers.

In another part of the NABIS project, a generic method for manipulating and transport of nanodroplets in a flow of a hydrophobic host fluid, with, has been developed. A completely automated instrumentation for flow-based microreactors was constructed. Basically, nanoliter volumes of aqueous reaction mixtures are injected into a flow of a non- reactive hydrophobic liquid (typically a fluorocarbon), and transported as a train of droplets through the system. One of the tested applications is amplification of DNA (via PCR). Earlier problems with cross-contamination between the individual droplets has been completely solved, and there is now a zero carry-over.
A fully operational prototype has been constructed, and validation on medical samples e.g. for detection of certain cancers is planned.

Several detailed protocols, suitable for assays on microarrays, have been established, which include sandwich immuno-assays and assays for autoimmune diagnostics. Preliminary tests of protocols for cell-assays, where the robotic system from KTH has been utilized, have also been carried out.

Another part of the work has been the design of optimized surface acoustic wave (SAW) devices, to be used for nano-agitation during assay incubation. The idea was to obtain an enhanced mass transport, thereby shortening the time required for an assay. This concept proved to be successful. Practical tests with protein chip assays have shown that the concept results in an improved efficacy. Compared to standard procedures, the use of SAW mixing led to an increase the 2-4 fold increase in signal intensity 2-4 fold, while standard deviations were significantly reduced (typically by a factor of 4).

Further, we have developed a technology for the preparation of microchips with a dense array of nano-electrodes. Figure 8 shows a prototype of such a chip.

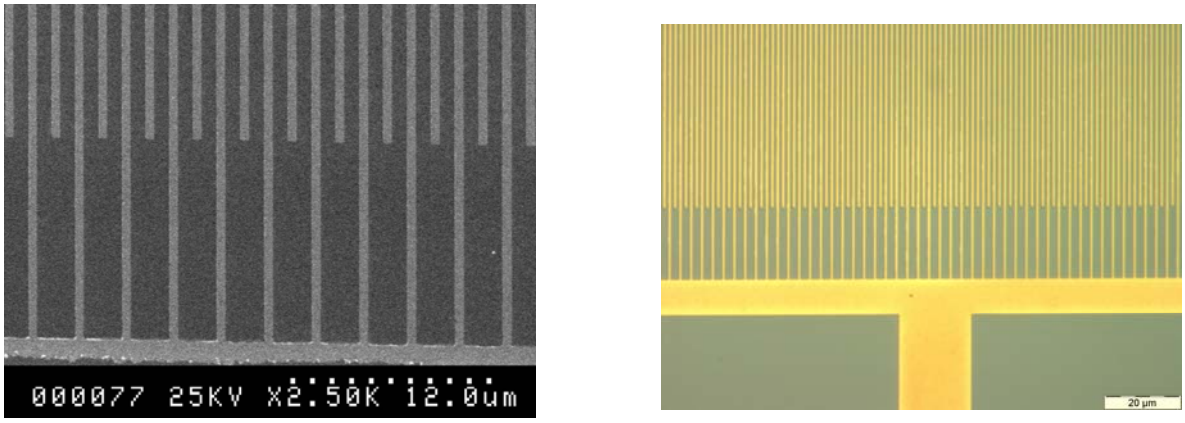

Figure 8. Photomicrography (right) sub micrometer inter-digital electrode of gold on a glass substrate, and (left) enlargement, showing 0.15µm line and 0.95µm spacing features.

Also, a complete instrumentation and software for immuno-assays, based on electrochemical detection combined with flow-through microchips has been developed. This system has shown an outstanding robustness and performance. It is now at an exploitation stage and future work will be focused on the development of new applications.

In conclusion, it can be noted that the long-term objective of the NABIS project is to obtain concepts and technology for improved biochips in terms of assay speed, sensitivity and dynamic range. Future applications are particularly in clinical diagnostics and drug development, but also in areas like food analysis and environmental monitoring. The results of the project will primarily be exploited by the partners themselves, particularly the SME´s involved. However, the NABIS project other entities for collaboration and further exploitation of our findings and know-how.

The project has a website, where addresses and useful links can be found.. The address of this website is: www. nabis.kth.se

Further information can be also obtained from the coordinator:

Prof. Johan Roeraade
Royal Institute of Technology (KTH)
Dept. of Chemistry, Analytical Chemistry
SE. 100 44 Stockholm, Sweden
tel: +46 8 790 8214, fax: +46 8 108425
email: jroe@analyt.kth.se

2. Dissemination and Use

Publishable results

The " Device and Composition for Droplet Microfluidics" is primarily intended to be utilized for DNA analysis (PCR amplification). The partners and owners of the IPR and know-how are CURIE/CNRS. More information can be obtained from:

Dr. Jean. Louis. Viovy, Institut Curie, Paris, France email: jean-louis.viovy@curie.fr

The proprietary ownership of the *SAW technology* is by Advalytix. and is protected by proprietary know-how along with a number of basic patents. Recently, Advalytix became part of Olympus-Europe. More information can be obtained from:
Dr. Christoph Gauer, Advalytix AG,, email: Christoph.gauer@olympus-europa.com

The flow chip assay concept, using miniaturized electrochemical detection method is exclusively owned by DiagnosSwiss SA. The system provides a high sensitivity and multi-channel capacity for various diagnostic applications. DiagnoSwiss is interested in new applications and possible partners for this purpose.
More information can be obtained from:
Dr. Frédéric Reymond, DiagnoSwiss SA, email: f.reymond@diagnoswiss.com

The multi-droplet handling platform, developed and owned by KTH has many generic applications, e.g. in combinatorial material science and chemistry. Collaboration for new applications and further research are invited. More information can be obtained from:
Prof. Johan Roeraade, KTH-Stockholm, email: jroe@analyt.kth.se