Publishable executive summary

Summary of the objectives

The goal of the NABIS research project; a three years effort which started in January 2004, is to study and develop novel synergistic technologies for development of the next generation of high performance biochips. These chips are anticipated to become very important in areas like accellerated drug discovery, diagnostics and personalized medicine. One of the key technologies relies on a predictable self-organization of fluids, where both static and dynamic formats are developed. The formats will be combined to overcome current bottlenecks in the automation of high throughput assays, and will also be used for the development of a new concept for automated chip replication. 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, are being 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. A third technology based on externally applied surface acoustic waves is also incorporated into the platform, yielding efficient agitation of nanodroplets. Finally, a nanoelectrochemical detection system will be developed. The technologies will be combined to yield an optimized biochip which will be tested using standard bioassays, and the results obtained 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	Naturwissenschaft-liches 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 wires.
* Grafting different chip surfaces with functional biomolecules is a 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 on the new biochips will be developed.
* In the final phase of the project the various technologies are combined in an iterative way to obtain an optimum performance.

Results achieved during 2005

Basically, all goals, which have been set for the reporting period, have been achieved.

Manufacturing protocols for structures, which are surface-enlarged with nanopillars have been developed for both in silica and plastics. Also, several technologies for surface hydrophobization have been developed, which greatly facilitates the confinement and self-organisation of bioactive molecules onto well- defined areas (dots), to be employed for bio-assays. Also, we have succeeded in making magnetic nanodots, both in array formats in into channels (fig 1).

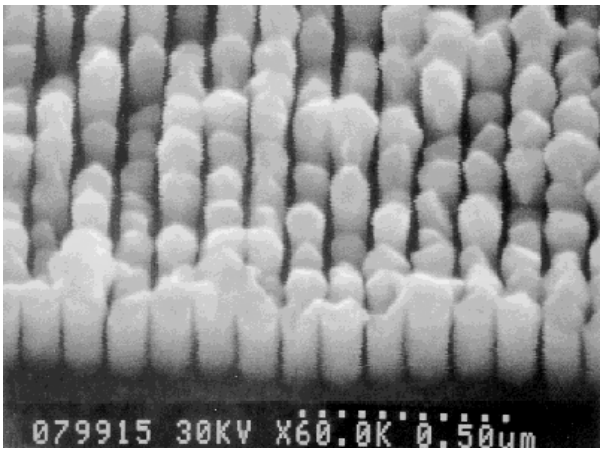

Figure 1. Scanning electron micrograph of nano-dot arrays formed by electroplating, defined by nanoimprint lithography.

The nanodots are used as anchoring points for functionalized magnetic nanoparticles, which then formnanowires. An example of long nanowires is shown in figure 2

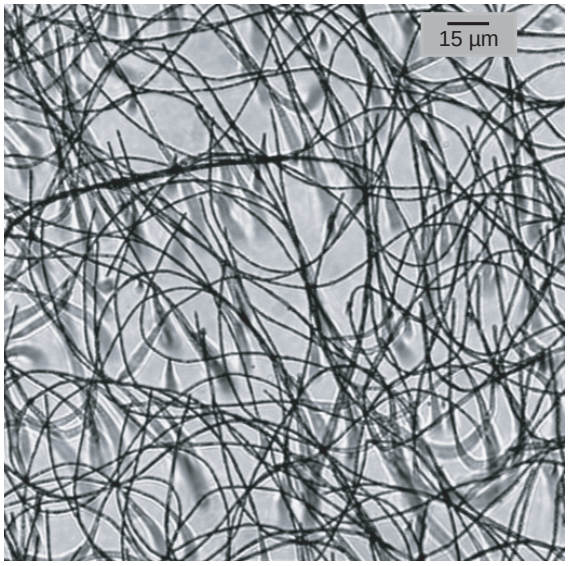

Figure 2: Photograph of long magnetic nanowires made by submitting a mixture of magnetic particles to a 25 mT magnetic field during 5 min. The chains are bent by gravity after field removal.

Grafting of polymers onto the wires and well as the surface-expanded structures by means of self-assembly is will on its way.

In another part of the project, we have developed an automated instrumentation for flow-based microreactors. 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 anticipated applications is amplification of DNA by means of PCR. Important progress has been made in terms of eliminating cross- contamination between the individual droplets, and we have applied for a patent for this principle.

Also, new concepts for automated spotting of both micro- and nanodroplets of liquid in various array formats have been developed. We are now able to handle extremely “sticky” protein solutions, which are known to be very problematic with conventional instrumentation like piezo-electric devices or contact spotters. Our system as a high sample capacity, typically 180.000 spots overnight. This capacity will be dramatically increased during the next working period of the project. Figure 3 shows an example of a microscopic slide, where 900 sample spots were deposited..

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

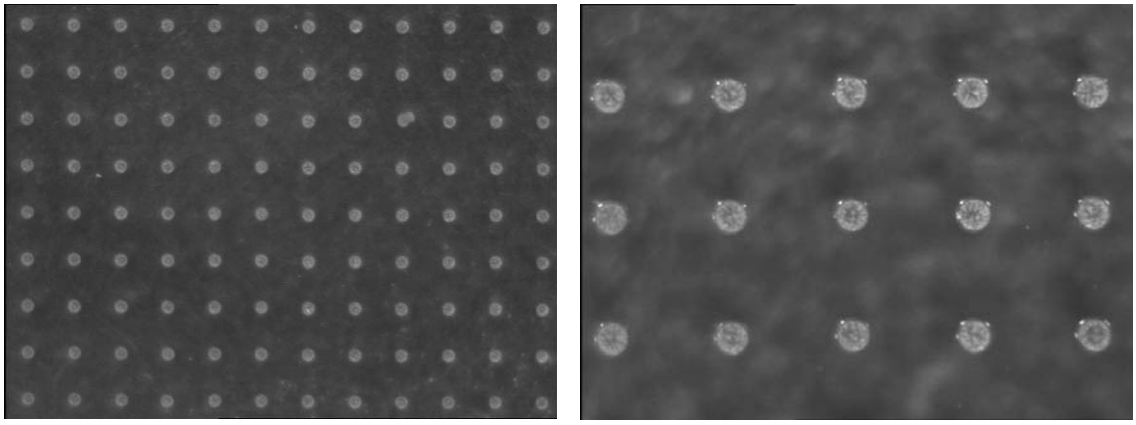

Figure 3. Left: Picture showing part of a 900 spot matrix after deposition of an anti-mouse IgG solution. Right: Magnification of part of the left picture.

The work has also included biofunctionalisation of the surface-expanded structures. Assay development on such structures is in progress. Some clear improvements compared to classical array structures have already been noted.

Another part of the work has been the design of a new surface acoustic wave device, to be used for nano-agitation during assay incubation. The idea is to obtain an enhanced mass transport, thereby shortening the time required for an assay. Important progress has been made in this area. First, a new generation of miniaturized instrumentation has been developed, with a more output power. Second, the first tests carried out with practical protein chip assays have shown that the concept results in an improved efficacy.

Additionally, we have now developed a microchip with a dense array of nano-electrodes, to be used in the next generation of electrochemical detection. Also, instrumentation and mathematical models for optimized use of the array system have been developed. In is anticipated that this will lead to improved sensitivity, thereby expanding the applicability of rapid, label-free immunoassays. Figure 4 shows a prototype of such a chip.

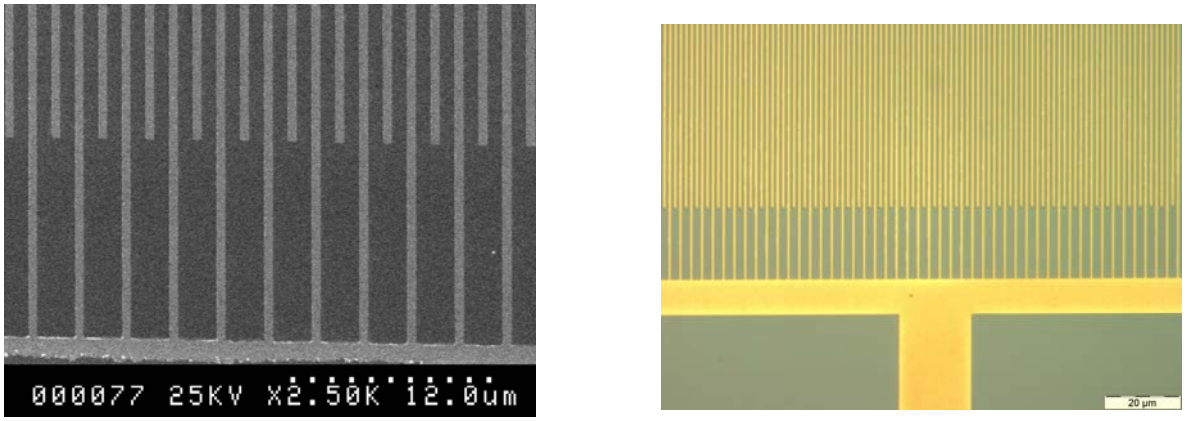

Figure 4. Photomicrography (right) sub micrometer inter-digital electrode of gold on a glass substrate, and (left) enlargement, showing 0.15µm line and 0.95µm spacing features.

Final goal of the project

The final 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, but also in developments of personalized medicine. The results of the project will primarily be exploited by the partners themselves, particularly the SME´s involved, or in the framework of possible new startups. However, the NABIS project also invites larger enterprises for possible agreements and/or collaboration, aiming at a large and global commercial exploitation of the results obtained.

The results of the project are (after protection of intellectual property), published in peer reviewed scientific journals and presented at international conferences. The project also organizes meetings and workshops, in order to further disseminate the results, and to obtain new inputs for progress of the work.

The project has a website, which is continuously updated with new, publishable results, useful links etc. The address of this website is: www. nabis.kth.se

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