TU/e technische universiteit eindhoven

Inaugural lecture
16 March 2007

prof.dr. Menno Prins

biosensors research – from molecule to system

/department of applied physics

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Presented on 16 March 2007
at Technische Universiteit Eindhoven

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prof.dr. Menno Prins

Introduction

This inaugural lecture deals with the research work that my colleagues and I are doing in the field of biosensors. As the Rector Magnificus mentioned, this speech marks the establishment of the chair 'Molecular Biosensors for Medical Diagnostics' at this University. I will lead you into the world of rapid molecular biosensors, tell you about the principles and challenges, and I will explain how we contribute to this field of research.

First let me give a definition of the word biosensor. A biosensor is an instrument that can rapidly measure the concentration of biological molecules in a fluid such as blood or saliva. Figure 1 shows a picture of the most commonly used biosensor: the glucose sensor, which is very important for people with diabetes. This sensor consists of two components, namely an electronic reader and a disposable cartridge with the shape of a strip. It works as follows. The user inserts the cartridge into the reader instrument. The reader recognizes the presence of the cartridge and asks for a drop of blood. Then the user pricks him or

Picture of a commercial glucose sensor. The sensor consists of an electronic reader which operates with a disposable strip-like cartridge.

figure 1

herself with a tiny needle in order to obtain a small droplet of blood on the skin. The user moves the droplet toward a small opening in the cartridge. When the droplet touches the opening it is drawn into the cartridge and comes into contact with materials inside the cartridge. This initiates a series of biochemical reactions and within a few seconds the instrument indicates what the glucose concentration is in the blood. The user then reads the glucose level and uses this information for an optimal administration of insulin.

The glucose sensor is a very good product: it is small, rapid, easy to use, reliable, and economical (every cartridge costs about 1 Euro). It fits into the everyday life of people with diabetes. Now you might wonder why biosensor research is needed if a high-quality biosensor already exists. The reason is that one would like to measure other substances than just glucose, for example hormones, drugs, proteins, and nucleic acids. These substances have concentrations that are orders of magnitude lower than glucose. So the challenge is to develop biosensors that are much more sensitive but that are still as small, as rapid, and as reliable as the glucose sensor.

It requires some imagination to realize how small the concentrations are that we want to measure. Let us do an experiment. We take two Olympic swimming pools. I blind my eyes while you throw a single millimeter-sized grain of material in either one of the two pools. We then leave the scene to allow the material to completely dissolve and spread out in the water. When we return we take a sample from each pool and determine the molecular concentration in both droplets. A good sensor is able to determine in which of the two pools you threw the grain.
The corresponding concentration is of the order of a nanogram per liter, which is one billion times lower than the concentration of glucose in blood. Such low concentrations can already be detected using large laboratory instrumentation. An aim of biosensors research is to demonstrate the detection of similar and lower concentrations, but now in a compact system, in a sample of just 1 microliter, and in a time of only 1 minute.

Biosensor products are part of the so-called *in-vitro* diagnostics market. This market includes instruments, materials and services that analyze human body fluids in order to evaluate diseases and other medical conditions. Total sales in this market are about 30 billion Euro per year [1]. About $^{2}/_{3}$ of the turnover is generated by laboratory testing and about $^{1}/_{3}$ by testing outside laboratories. The trend is that more and more testing occurs outside laboratories. Important reasons for this trend are that professionals want to improve the effectiveness and efficiency of their workflows, and that consumers find benefits by testing in their daily life.

Rapid biosensors can be applied in many domains, e.g. medical, veterinary, food, safety, forensic and environmental applications. Let me give three concrete examples of future applications of biosensors. A first example is in the medical domain. There are a number of low-concentration blood proteins that are indicative of the condition of the heart. Diagnostics and monitoring of heart diseases will improve if medical doctors and individual patients can rapidly and easily measure these proteins. A second example is in pharmaceutical treatment. It is known that patients can respond very differently to pharmaceutical drugs. When a drug is taken at a certain dose, it may work well in some patients while it is ineffective or gives harmful side-effects in other patients. Therefore, it is expected that patients will use biosensors in the

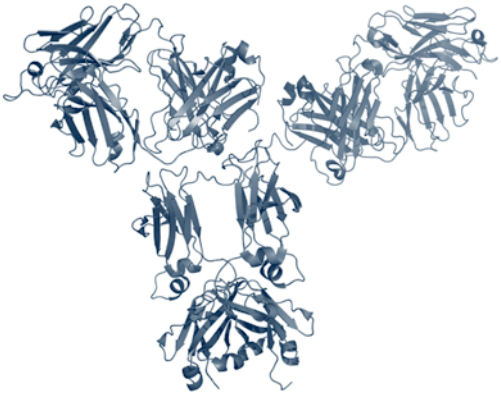
future, in order to adjust the medication intake to their personal need. A third example deals with road safety. An important cause of traffic accidents is the use of substances that affect driving ability. Alcohol consumption has long been the biggest problem, but fortunately the number of alcohol related accidents has gone down due to a combination of regular educational campaigns and regular alcohol checks at the roadside. Nowadays however, the number of fatal accidents caused by the use of illegal drugs is rising. As a consequence there is a great demand for a biosensor suited for rapid drug testing at the side of the road.

These examples involved the detection of proteins and drugs, two important classes of biological material. There are other classes of materials that are also interesting for rapid-testing applications. One example is the detection of biological cells, for example bacterial cells or white blood cells. Another example is the detection of DNA, the genetic material inside a biological cell. There is a trend of steadily increasing knowledge of how molecules and cells function in the human body due to worldwide biomedical studies. Such studies regularly generate new markers, i.e. specific molecules that give information about health and disease. New markers will generate new applications for biosensors, which is another reason why biosensors research is an important investment for the future.

Molecular biosensors and the system challenge

In the following section I want to delve into the technology of molecular biosensors and describe the research challenge from a systems perspective. In this presentation I will focus on protein-based sensors because in this field we expect that our technology investigations will have most impact in the near future.

A topic that requires special attention is the complexity of biological samples. Let us assume that we want to detect molecules of type 'A'. In biological samples, the molecules 'A' are surrounded by high and variable concentrations of many other biological molecules. This implies that a biosensor needs to be very specific. We need some kind of selection principle to fish out specifically the molecules 'A' from the complex fluid. Fortunately nature gives us a helping hand with molecules called antibodies, sketched in figure 2. Antibodies are large proteins with strong binding properties. The immune system of living beings constantly produces antibodies with the purpose to catch unwanted intruders such as



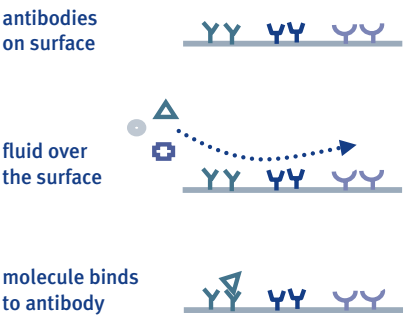
Sketch of an antibody, a protein with very strong binding properties. Antibody molecules have a characteristic Y-like shape. The size of the molecule is about 15 nanometers.

figure 2

harmful bacteria and viruses. The foundations of antibody-based tests were laid in the 1960's [2]. Antibody technology has significantly progressed, and nowadays one can purchase antibodies against many different molecules. This is very useful for biosensors research, because we can make a biosensor for molecules 'A' by providing a surface with anti-A antibodies. Such a biosensor is called an immuno-sensor. When this surface is exposed to a sample fluid, the molecules in the fluid will bounce against the surface, and molecules of type 'A' will be specifically caught by the anti-A antibodies (see figure 3).

The surface with antibodies is a central part of the biosensor, but it is not enough. To build a complete biosensor, a sequence of process steps is needed:

- *Sample pretreatment*. A sample normally needs some kind of pretreatment. For example it is passed through a filter, or (bio)chemical substances are mixed into the fluid.
- *Transport*. After pretreatment, a transportation process is needed to bring the molecules toward the biosensor surface and into contact with the antibodies.
- *Specific binding*. When the molecules reach the surface, conditions need to be created to enable rapid biological binding between molecules 'A' and the antibodies on the surface. The binding should be specific and non-specific binding to the surface should be minimized.



Top panel:
A biosensor surface with antibodies.
Middle panel: Fluid flows over the sensor surface.
Bottom panel:
An antibody captures a molecule from the fluid.

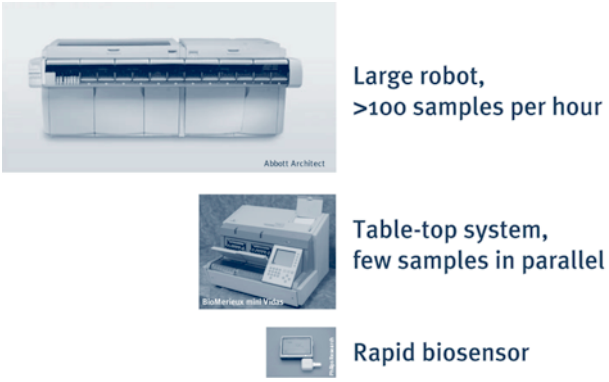
figure 3

- *Detection*. Finally the system should detect how many molecules of type 'A' have been bound to the surface.

Now we can define the challenge of biosensors research: the challenge is to create a compact system that integrates all required process steps and that improves the key performance parameters: sensitivity, specificity, speed, accuracy, dynamic range, robustness, ease of use, costs.

Let us take a historical perspective. When antibody-based tests were first performed in the 1960's, a total test involved a long list of manual procedures that needed to be performed in a laboratory. The total test took many hours and errors were often made due to the many manual steps. In the following decades robotized instruments were developed for automated fluid handling and the simultaneous processing of many samples. Such robots are presently used in all clinical laboratories for high-volume blood testing (see figure 4).

Parallel to the development of large robotized laboratory instrumentation, technologies are being developed for rapid testing outside laboratories. Products presently on the market are mostly based on so-called immunochromatography [2]. The search for improved biosensors has led



Different sizes of instrumentation: A large robot (top), a table-top system (middle), and a palm-sized biosensor (bottom). Sources: Abbott Diagnostics, BioMerieux, Philips Research.

figure 4

to the research field called *lab-on-a-chip*, a laboratory-on-a-chip. The word 'chip' refers to a miniaturized system, because computer chip technology is a prime example of miniaturization. In the 1990's the first university groups started to apply miniaturization techniques to the analysis of fluids. In the meantime several groups have demonstrated that it is in principle possible to perform fluid analysis in a miniaturized device. However, it also became clear that lab-on-a-chip devices often only work properly in a well-controlled environment with well-controlled fluids and operated by well-trained users. For a breakthrough new biosensor technologies are needed which – in addition to being sensitive, specific, and rapid – are able to cope with variations in environmental conditions, can deal with variabilities in biological samples, and are reliable in the hands of unskilled users.

Research examples with magnetic particles

In the previous section I described that the challenge of biosensors research lies in the development of a complete system. In practice we conceive a system, subdivide this into modules, and then formulate research projects on specific elements of the system.

We have chosen to initially focus on biosensor concepts employing magnetic particles. Magnetic particles are well known in biological analysis, for example for the extraction of cells from a sample [3]. The particles bind to the cells and are then rapidly extracted using magnetic fields. This concept is robust because biological material in itself is hardly magnetic. We want to further extend this line of thinking and use magnetic particles also for other key processes in a biosensor, with the aim to create a biosensor system that is rapid and robust.

Picture of magnetic particles taken with a scanning electron microscope (SEM). On average the particles have a diameter of 300 nanometer. In our research we study particles in the range between 50 nanometer and 1 micrometer.

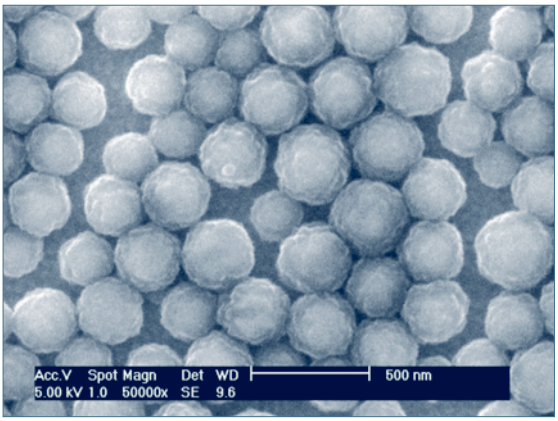
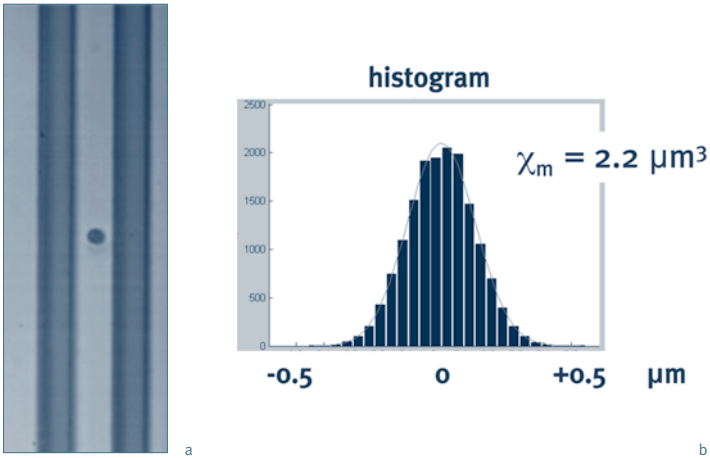


figure 5

The basis of our work is the magnetic particle. Figure 5 presents an electron-microscopy image of magnetic particles with a diameter of about 300 nanometer. These particles are so-called superparamagnetic particles [3]. They internally contain many ferromagnetic grains with a size of 5-15 nanometer. The grains are so small that they quickly lose their magnetic moment in absence of an external magnetic field. Superparamagnetic particles are readily magnetized to large magnetic moments, yet the mutual magnetic attraction between different particles can be switched off, preventing irreversible aggregation.

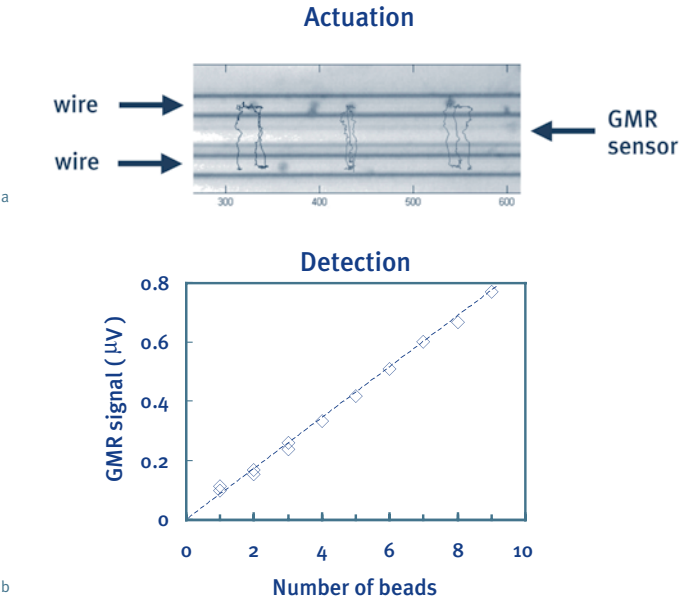


Experiment with a single magnetic particle. Panel (a) shows an optical image of a particle on a chip surface with a wire. Panel (b) shows a histogram of particle positions on the surface. The histogram reveals the magnetic susceptibility of the particle [4].

figure 6

Figure 6a shows an optical image of a particle that was suspended in a fluid and placed on a chip surface with a wire [4]. In the corresponding movie we observed that the particle showed irregular movements caused by the thermal energy. All materials vibrate to some extent due to thermal energy and such vibrations are particularly visible for small objects. In spite of the irregular motion we observed that the particle stayed in the vicinity of the microfabricated wire when the wire was powered with an electrical current. From histograms of observed particle positions (see figure 6b) we conclude that the confinement of the particle to the wire is caused by the magnetic potential well generated by the

electrical current and the magnetism of the particle. The crux of this experiment is that we can derive the magnetic properties of an individual nano-particle from the observed irregular motions on a wire.
This represents a completely new approach in this field of research.



Panel (a): Optical microscopy image of a sensor chip with two current wires and a GMR (Giant Magneto Resistance) sensor [5, 6]. The traces of three particles are shown, hopping between two wires. The traces were reconstructed from a series of images taken with a high-speed camera. The three particles crossed the GMR sensor due to alternated powering of the wires with an electrical current. Panel (b): The GMR sensor signals recorded as a function of the number of hopping magnetic particles (X.J.A. Janssen *et al.*, to be published).

figure 7

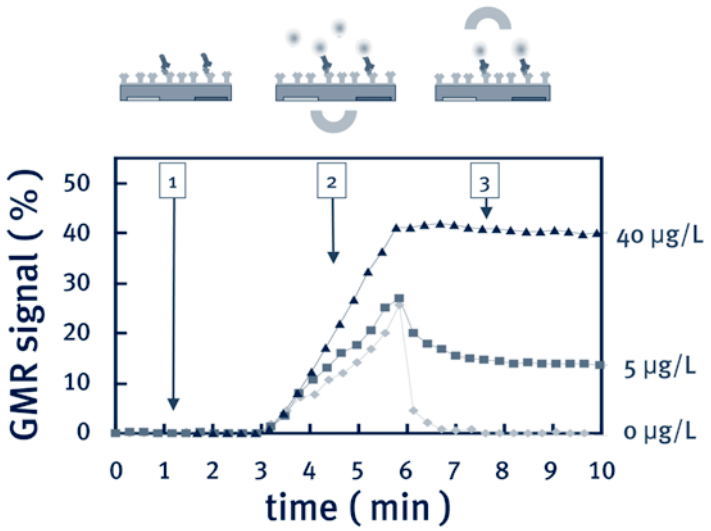
In another experiment we studied the controlled movement of a magnetic particle on a surface by using multiple wires on a chip. Figure 7a shows the traces of three magnetic particles that hopped between two wires. The wires were alternately powered by an electrical current. During the hops of the particles we recorded their positions by a high-speed camera as well as by a magnetic sensor embedded in the chip. The sensor was a GMR (Giant Magneto-Resistance) sensor [5, 6], made from material that strongly changes its electrical resistance when exposed to a magnetic field. Figure 7b shows an experiment in which the number of hopping particles was varied. The data show that the measured signal scales linearly with the number of hopping particles and that single particles can be detected.

After these experimental demonstrations of the monitoring and control of individual magnetic particles on a chip surface, we can consider the possible roles of magnetic particles in a biosensor system:

- *Sample pretreatment*. Magnetic particles can be used to agitate and mix fluids [7].
- *Transport*. Magnetic particles can be used as carriers that transport biological molecules toward the chip surface.
- *Specific binding*. Magnetic forces can be applied to the magnetic particles to generate rapid as well as specific biological binding to the chip surface. For example, forces can be applied to concentrate particles at the surface, and also to remove weakly bound particles from the surface.
- *Detection*. Magnetic particles can be used as so-called labels, indicating the presence of the molecules of interest on the sensor surface.

The above-mentioned functions all generate challenges and scientific questions. The mixing and transportation processes raise questions in the fields of multi-particle dynamics and fluid dynamics. The binding process requires an understanding of the combined physical, chemical and biological forces that appear when a small particle is near to a surface. Finally, the detection process raises questions about signal and noise in the physical and electronic domain.

Figure 8 presents an example of an immuno-biosensor experiment in which several of these processes occur. Three traces are shown from three experiments with different concentrations of a small protein called



Time-trace of a biosensor experiment with magnetic particles. Particles near the chip surface were detected using giant magneto-resistive (GMR) sensors embedded in the chip [5, 6]. Three traces are shown from three experiments with different concentrations of parathyroid hormone (PTH), namely 0 µg/L, 5 µg/L, and 40 µg/L. In step 1, PTH molecules were bound to the chip surface and sandwiched between two anti-PTH antibodies. In step 2, magnetic particles were attracted toward the chip surface by magnetic forces and were allowed to bind to the surface. The number of bound magnetic particles became a measure of the number of PTH molecules on the chip surface. In step 3, unbound and weakly bound magnetic particles were pulled away from the chip surface by a magnetic field. The signals clearly depend on the concentration of PTH (W.U. Dittmer *et al.*, Philips Research, to be published).

figure 8

parathyroid hormone (PTH). The sensor was a silicon chip containing GMR sensors, with a surface coated by anti-PTH antibodies. The graph shows the signal measured by the on-chip GMR sensor as a function of time, indicative of the number of magnetic particles present on the chip surface. The experiment was performed in three steps. In the first step, PTH molecules were bound to the chip surface and subsequently tagged by a second type of anti-PTH antibody. The result is a sandwich format in

which every PTH molecule is sandwiched between two anti-PTH antibodies. In the second step, magnetic particles were attracted toward the chip surface by magnetic forces. These particles had a surface coating with specific binding properties to the second type of anti-PTH antibodies. In this way the number of bound magnetic particles became a measure of the number of PTH molecules on the chip surface. In the third step the unbound and weakly bound magnetic particles were pulled away from the chip surface by a magnetic field. The important point of this series of experiments is that the size of the GMR signal scales with the concentration of PTH, indicating that the device functions as a biosensor.

This closes the section on experimental approaches in our research, with the focus on the use of magnetic particles. I have described how we can control and observe the dynamics of individual magnetic particles on a chip surface, and how we can use magnetic particles for biological detection. In the future we will study the detailed dynamics of magnetic particles near a biological surface, we want to study the properties of individual biological bonds on a chip surface, and in addition to detecting the presence of biological molecules we expect to be able to determine biological functionality inside a biosensor system.

We investigate and use particles that are small enough to be substantially affected by biomolecular forces, and large enough to be controlled and detected by electromagnetic principles. The biological molecules have a size ranging between 1 nanometer and 20 nanometer, whereas the particles have a size ranging between 50 nanometer and 1 micrometer. The particles have a size that is intermediate between the biological molecules and the larger biosensor system. One can view the particles as the nano-scopic agents that help us to penetrate into the biomolecular world.

Way of working

The presentation has predominantly dealt with technical topics. However, the way we approach our work is equally important. I would now like to say something about multidisciplinarity, collaborations, education, and about working in a group.

Biosensors research involves several scientific disciplines: electronics – mechanical engineering – physics – chemistry – biochemistry – medical biology.
The logic in this sequence lies in a gradually changing scientific approach. The first disciplines in the row often have detailed descriptions available of the basic mechanisms. The tendency is to take a device or systems perspective and to apply research methods such as design and modeling. The last disciplines in the row still have very limited quantitative model descriptions available. The molecular perspective is the basis of the work, and studies are mostly empirical.

Worldwide, many groups are active in the biosensor field. Some groups emphasize the device perspective and focus on system integration and miniaturization of transportation principles. Leading themes are electric-field driven transport and the miniaturization of mechanical elements. Other groups emphasize the molecular perspective and focus on the development of new molecular detection tools, for example mixed immuno-DNA technologies or detection based on cell biochemistries. It is crucial to link these two approaches. In our work the link is attained via the magnetic particles, which we use for materials transport as well as for molecular detection.

To reach top-level results one needs to establish collaborations. These come in two different types, namely as partnerships that combine different disciplines and partnerships that combine different technology maturity levels. Table 1 indicates a few parties that are involved in our present collaborations. Interdisciplinary partnerships aim at bringing all required disciplines to the table and at bridging the device and molecular perspectives. Cross-maturity collaborations link scientific understanding

Parties with different backgrounds are needed for collaborations in biosensors research, combining different technological disciplines and different technology maturity levels. Some of our collaboration partners are mentioned in the table.

		Technology discipline	
		Physics Chemistry Electronics	Biochemistry Medical Biology
Technology maturity	Academic	Univ. Eindhoven, ...	Univ. Wageningen, Univ. Maastricht, ...
	Company	Philips, ...	Future Diagnostics, Cozart Bioscience, ..

table 1

with potential products. For example, in the present collaboration between TU/e and Philips Research, the University group focuses on basic concepts, new principles, education, and publications, whereas Philips Research investigates technology integration and product-oriented solutions.

Collaborations do not come easily. It requires energy to explore an interface and build a partnership, but it is extremely rewarding when a collaboration appears to be fruitful. I always keep in mind that what is an interface today may become a new field of technology or a new company in the future.

The multidisciplinarity of the biosensors field sets some special demands to the education system, particularly in finding a good balance between depth and breadth. In the first years of studies the focus should be on depth – students should learn the basics of science, learn to sharply analyse scientific problems, practice experimental skills and understand how to develop model descriptions. In a later phase breadth can be added – students should be offered opportunities to broaden their views and to learn about other scientific disciplines. The department of Applied Physics is well-positioned for these requirements. Physics has a strong focus on quantitative understanding and system analysis. Excursions can

be made into other disciplines such as chemistry and biochemistry, e.g. by performing a broadened Minor during the Bachelor phase. In the Master phase, students can perform studies at the physics-chemistry interface in several research groups. The group on Molecular Biosensors now gives students a possibility to perform studies at the physics-chemistry-biochemistry interface. We hope to continue this trend in the new bio-nano-technology chair that is being defined in the framework of the 3TU federation, and via links to the growing chemical-biochemical activities in TU/e.

During their education students learn how to address problems in a scientific way. The first problems they get can be solved in a short time, e.g. during the time of an exam. In the course of the studies they learn to deal with problems of longer and longer timescales. Master students stay in the group for 9 months, PhD students stay for a period of 4 years. Increasingly we learn the students how to deal with uncertainties, and how to strike a balance between trying something new and building on known approaches. Good science is a mix of old and new, and has elements of creativity and of thorough analysis. I very much enjoy the science and technology profession for the combination of creativity and analysis.

Another aspect that motivates me in my professional life is the interplay between group and individual. The majority of our activities and experiences occur in relation to fellow beings, certainly when working in a multidisciplinary research group. It is important to learn to deal with the duality between group and individual. The art is to be part of a group while contributing to it as an individual. One aspect of the challenge is to connect to a group, another aspect of the challenge is to stand as an individual. It is important to pay attention to such processes because these determine how people can grow both individually and in the team. One possible way to contribute to a group is by presenting oneself and one's work, which is one of the reasons why this inaugural lecture is special for me.

Acknowledgments

Finally, ladies and gentlemen, I would like to thank a number of people.

In the University I want to thank Wim de Jonge and his successor Klaas Kopinga for giving me the opportunity to establish the research group Molecular Biosensors for Medical Diagnostics in the department of Applied Physics. Furthermore I express special thanks to Leo van IJzendoorn, Arthur de Jong and the other members of the research group for their enthusiasm to jointly undertake this endeavor. It is an honor to be part of the University and I always enjoy the lively and sharp discussions with students and colleagues from several departments.

At the University I have a part-time assignment. In parallel I am a scientist at Philips Research, where the goal is to bring new technologies to the market. This requires collaborations between technology experts, application experts and business experts. I feel privileged to participate in such a process and I have respect for the people who fund the long routes of technology innovation. I want to thank Loek Nijman, Bertus Pals, Henk van Houten and Hans Hofstraat, managers who have given me support during several years of biosensors research. Furthermore I am very grateful to my colleagues in Philips, inside and outside the magnetic biosensors team, who contribute one way or another to movements of Philips into the biomolecular domain.

I thank our biotech partners for the fruitful collaborations, particularly Future Diagnostics and Cozart Bioscience. We have learned to understand each other's languages and your commitment has been instrumental for the progress that we are jointly making.

I also want to thank my family and friends for their years of support and their encouragements to explore new things. My parents, brothers and sister, you have laid the basis for what I am; I am very happy that you are here today. Cilia, we share an interest in bio-medical topics albeit from very different angles. Thank you for your love and your presence, and for the joy of jointly taking care of Victor, Floris and Rosa. Victor, you and

I have become regular visitors of the TU/e annual open days together with your grandmother, a beautiful example of how science can bring people together at all ages.

Finally, when I see that I am at the interface between several scientific disciplines, at the interface between a university and a company, and in the midst of creativity and analysis, I realize that I am in a unique position. In this position I look forward to develop excellent science and technology with the students and staff at Eindhoven University of Technology.

I thank you very much for your attention.

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Curriculum Vitae

Prof.dr. Menno Prins has been appointed part-time professor at Eindhoven University of Technology (TU/e) for Biosensors Research in the Department of Applied Physics as of 1 March 2005.

Menno Prins holds an M.Sc. degree in 'Physique et Applications' from Université Paris XI, and an M.Sc. degree Applied Physics from Delft University of Technology (cum laude). He performed a half-year research visit at the Indian Institute of Science in Bangalore.
He received a Ph.D. degree in Experimental Physics from University of Nijmegen, where he studied magneto-optical scanning tunneling microscopy. Since 1995 he works in Philips Research, first in the group Integrated Device Technologies and presently in the group Healthcare Devices and Instrumentation. Earlier topics of research were thin-film oxide transistors and fluid control in microchannels by electro-wetting. Since 2000 his research is focused on molecular biosensors.

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