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AFM Based Manipulation for Mobile Diagnostics

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Abstract: Enormous Research studies in medical field, genetics and other process monitoring technology go together hand in hand with the latest advancements in nano-engineering, further more advancements in engineering and technology, control systems, robotics and automation, on the nano scale has enabled so far impossible studies and research in molecular biology and cellular biology, this has led to the various design and developments of new method's and setups which have further more extended the boundaries for the possible experiments in handling and manipulating bio-materials. In this paper the latest research work on two important areas have been focused - robust and accurate automation process to use the latest AFM technique for industrial processes a possible approach to design and structure bio-components from several nano meter up to a few micrometres. Mainly first the simulations and principles for the design of a new signal acquisition unit are shown, to set up diagnostic systems without complex optical setups or fluorescence dyes have been addressed in this paper.

Keywords: AFM; Cellular Biology; Mobile diagnostics; Nano meter

I. INTRODUCTION

Over the last years, the handling and manipulation of biomaterials started to become a focus research area of engineers. Up to this time, this part of research was only interesting for scientists in areas like medicine, biology, genetics or process monitoring. However, starting with the idea to use novel approaches for sophisticated medical treatments such as high-throughput characterization of bacterial cells, parts of cells and biomarkers, new methods for in situ characterization of biological processes or organs, as well as for new generations of process monitoring systems such as miniaturization and design microsystems and smart miniaturized systems and the use of alternative renewable raw materials such as DNA, cells or CNTs to build electric circuits to support the design and capabilities of biosensors, the development of new tools and methods for the handling and manipulation of biomaterials on the nano and micro scale were necessary.

In this paper we present our latest results of the development of nanorobotic systems and methods to allow unique experiments to examine cell components (DNA, wooden fibers), single cells (bacterial cells, blood cells) up to complete cell compounds (bacterial biofilms) (see Fig 1). these nanorobotic systems and methods, it will be possible to characterize the mechanical and electrical properties of biomaterials as well as to handle

and manipulate biological objects. We will introduce the current work on the structuring of biocomponents of biosensors and the design of a new signal acquisition unit for fluorescence-less measurements.

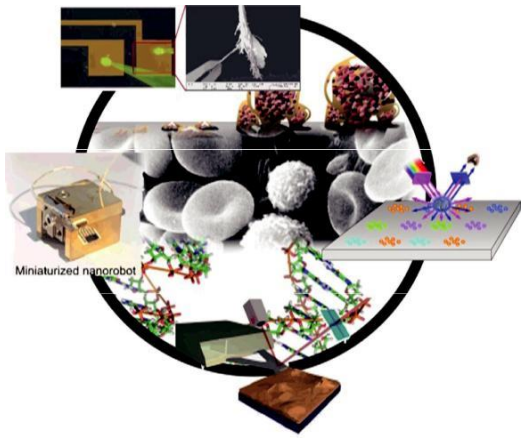

Fig 1. Schematic overview of the robotic systems and methods presented in this paper.

In the following sections, we introduce a method to for the automation of AFM based manipulation and handling methods for biological objects on the nano scale (Section II). In section III the structuring of biocomponents of biosensors is shown. Section IV gives an overview of first simulations and principles for the design of a new signal acquisition unit, to set up diagnostic systems without complex optical setups or fluorescence dyes. Finally, the paper is concluded in Section V, including a short outlook.

II. AUTOMATION OF AFM-BASED NANOMANIPULATION

When the AFM is utilized as a tool for nano manipulation of biological objects and materials, the AFM tip is used as an end-effector and the force sensing capabilities of the cantilever-photodiode-system are exploited to gain feedback of the manipulation process. However, it should be denoted that the AFM is equipped with only one cantilever, and together with the lack of an additional real-time visual sensor capable of observing nano scale objects, AFM-based manipulations thus have to be performed in a nearly blind way; only force data from the measured cantilever bending can give some sparse information about the success or failure during the manipulation. The serial nature of the AFM also reduces throughput of manipulations. To check for the success of manipulation, AFM scans are usually performed within the interesting region between single manipulation steps. This however requires a time and may also become difficult if the AFM tip requirements are different for imaging and manipulation.

To increase throughput, reliability and repeatability of AFM-based manipulations, several issues have to be addressed in order to reach the final goal of a fully automated AFM-based handling system.

Major causes for high error rates in AFM-based manipulations are spatial uncertainties present in any AFM system. These uncertainties are partly induced by creep, hysteresis, and other nonlinearities of the AFM scanner. However, these effects can be compensated in recent commercial AFM systems/ scanners, most of them use feedback approaches where positioning sensors are utilized to operate the scanner in a closed loop.

More critical and less straight-forward to counteract are spatial uncertainties introduced by the effect of thermal drift. Due to thermal expansion of the different parts of the AFM, a time-variant displacement between AFM tip and sample occurs. In order to provide a flexible, fast, and sample independent drift compensation technique, a particle filter based drift measuring approach was developed and presented [1].

Automation of AFM-based manipulations is still at a very early stage and solutions have to be developed allowing for an easy adaption to any specific manipulation scenario.

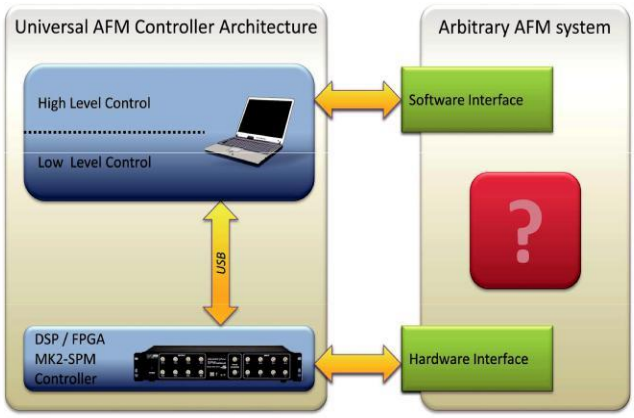

Fig 2. Scheme of the developed AFM control architecture.

To realize automation of AFM-based manipulation and to allow for interchangeability of different hardware platforms, a flexible AFM control architecture was developed (depicted in Fig 2). In most commercial AFM systems, high level control is available by a specific software interface (e.g. library functions or scripting). Controlling the AFM on a low level (e.g. real-time monitoring of the channel data, arbitrary tip movements), however, is often not feasible since low level control is usually implemented on a dedicated “black box” DSP system that does not offer any interaction through other software systems. Therefore, the developed control architecture (implemented in C++/C) is divided into a high level control part that communicates with the AFM’s SW interface and a low level control part that communicates with a custom-made DSP system. The high level control consists of an abstract interface and device specific implementation of all necessary control issues. The low level control communicates via USB with a DSP/FPGA based controller (implemented in

C/Assembler) that provides all basic AFM functionality (e.g. scanning, PI-controller for maintaining a constant force or amplitude, absolute and relative movement, and data transfer of multiple channel data etc.). The system has eight analog input as well as eight output channels that can be connected to almost any AFM system and thus providing a flexible means for replacing the original and often inflexible controller the AFM is equipped with by the manufacturer. To allow for more sophisticated experiments, different manipulation functionality can be added easily. First successful experiments using the developed control system were conducted on a commercial AFM system (JPK Instruments, Berlin, Germany). To prove the flexibility of the developed control architecture, it will be next tested in the AFM-inside-SEM setup that is described in detail in [2].

III. AUTOMATED STRUCTURING OF BIOMATERIALS

A big market in the process sensor industry deals with the development of biosensors. For a proper use the biological components of the biosensor have to be structured as fine as possible. Biosensors himself consist of a sensitive biological component, a transducer or detector element, and associated electronics or signal processors [3-5]. With an improved fine structuring of these components the signal enhancement can be improved (in case of SPR measurements via gold particle enhancement), the possibility to get multi information can be enabled (multi PCR analysis for forensic and clinical use) and the accuracy of the measured date can be improved (more samples of the same type for proper statistical analysis). One solution to deal with these points is the increasing of the possible density of biological components or components for signal enhancement on a discrete sensor surface.

Currently most micro fluidic approaches (nano printer, nano plotter, touch less printing) or µ-contact printing approaches (stamping technologies) as well as dip-pen lithography methods are used. To overcome the limitations of these technologies we examine the use of novel nano lithographic approaches also useable for microscopic biomaterials. By using the AFM it is possible to functionalize extremely small structures or remove small areas out of bio-molecular mono layers to enable binding sites for gold particles, biomarkers and single stranded DNA chains (for the detection of mutations of the DNA which can result in cancer) as well as for proteins, antibodies or complete cells. The goal of our work is the development of a fully automated method to manipulate biomolecular mono layers to establish a method for the structuring of biosensors.

Figure 3 shows an example of an APS monolayer with the scratched AMiR Logo. The width of the scratches is about 20nm and the height between 0.8 and 1nm, meaning that the APS monolayer was removed completely. As a rough estimation, using the values of these experiments, the density of small biomarkers like single stranded DNA, proteins or antigenes on the biosensors can be increased about a factor of at least 1*103 to 2*103. To reach this goal the processes are completely automated using the methods described in section IV. However, to establish a method with a broad range of use, it is necessary to enable the structuring also for bigger biomarkers like antibodies or small cells.

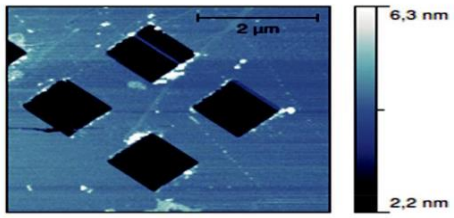

Fig 3. Height image of 1x0.5 µm AMiR logo scratched into APTES monolayer by AFM machining.

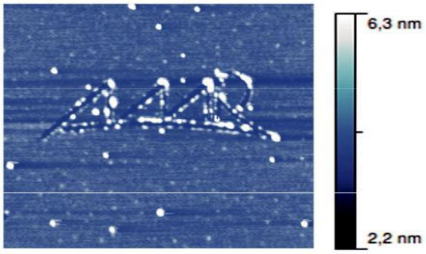

Fig 4. Height image of 1x1 µm area scratched into APTES mono-layer by AFM machining.

The two visible machining depths arise from different force set-points used during the processing (left) and AFM height image of a series of 1x1 µm sized scratched areas (right). In Fig 4 an example for sizes of about 1µm2 is shown. As can be seen, the structuring is possible as well, but the main problem arises in the contamination of the AFM tip because of the removing of a high amount of material from the surface. To overcome this problem future work is necessary dealing with the cleaning of the AFM tips to enable an industrial feasible use of this method.

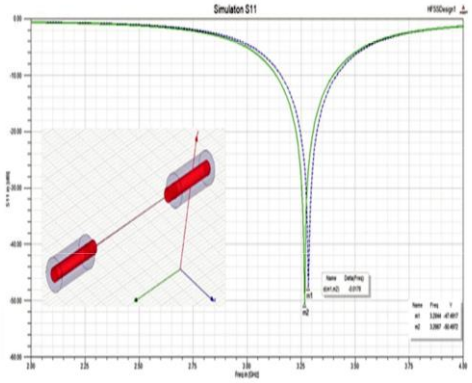

Fig 5. Micro fluidic-Resonator: RF-Simulation results.

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IV. SIGNAL ACQUISITION SYSTEM

In the following the Signal Acquisition Unit (SAU) of the proposed system will be depicted. The aim is to measure the dielectric properties in the microwave region of the EM spectrum of each Biosensor element as a function of frequency. 3D full wave EM modeling (done with an soft HFSS™) shows a dependency of the resonance characteristics by variation of the permittivity (see Fig 5). The structured sensor elements will be probed by electrodes in a coaxial, unsymmetrical manner.

The main sensing element (System Impedance = 50Ohm) consists of a resonator, shown in the lower left corner, which peaks at 3.284GHz. When biomarkers will be flowing through the dielectric, the resonance frequency of the resonator will change. In the EM-simulations, the frequency shift will be in the range of about 17MHz, when the structure will be disturbed by one single 20 micron sized object. This object is a sphere filled with distilled water. The resonance frequency shifting caused by the biomarkers will be detectable by a Spectrum Analyzer or Network Analyzer (NWA) its high sensitivity to small parameter changes is the nature of this kind of sensing structure. This means that even a few of immobilized biomarkers should be detected.

The block diagram (Fig 6) gives some hints about the proposed Signal Acquisition Unit with the exchangeable Biochips. These Biochips will be connected via Ball Grid Interconnects to the SAU.

The Controller-PC´s main task is collecting the reflection coefficient data S11, measured by the NWA, calculating the impedance mismatch caused by each Biochip and the Multiplexing Unit (MUX) and depending on this mismatch con- trolling the Impedance Matching Unit for S11=0 @ fres. This calibration works only under the assumption that the on-board TRL calibration standard is properly connected via the BGA-Connector and the MUX-Unit is switched to the CAL-Channel. Each Biochip has to be calibrated before final measurements could take place. The Device Under Test (DUT 1..n) in the here proposed layout connotes the AFM-structured substrates with the different biomarkers which should result in various frequency shifts.

The current lab setup for these measurements is shown in figure 7.To evaluate the functionality of the simulated resonator structure, a micro fluidic breadboard system was build. The prototype is made up of two sections: a micro fluidic and a microwave section. The Interface-Controller (I/F) controls the syringe pump and the 3-port-valve to transport different test fluids from a reservoir through the microwave resonator to another reservoir. The PC controls the output of a Rhode & Schwarz Signal generator and reads the data of a high-accuracy Tektronix Voltmeter which in turn measures the output of a logarithmic RF detector working in the resonance frequency regime. This reduced test setup was chosen in terms of industrialization of the SAU Previous works like [7] concentrates on spectroscopy methods of human cells in the region of 15GHz to 30GHz and this resulting in much higher frequency shifts of around 400MHz. One goal of this research in future is an industrialization of such a system. Keeping this in mind a) the frequency range should be lower than 4GHz to overcome problems resulting in such high frequencies domains and b) the whole lab setup has to be exchanged by on-chip components which could be controlled by a microcontroller.

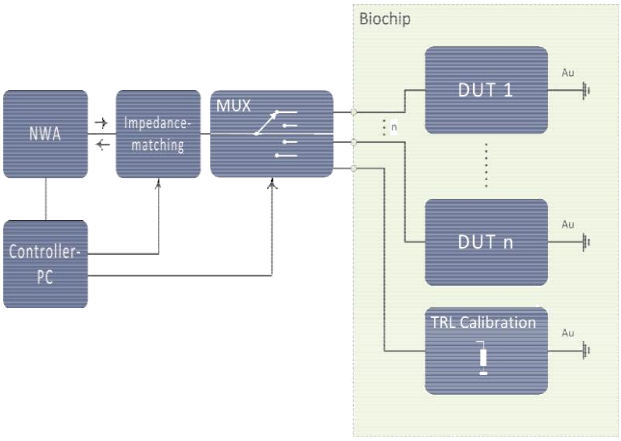

Fig 6. Biochip SAU Layout.

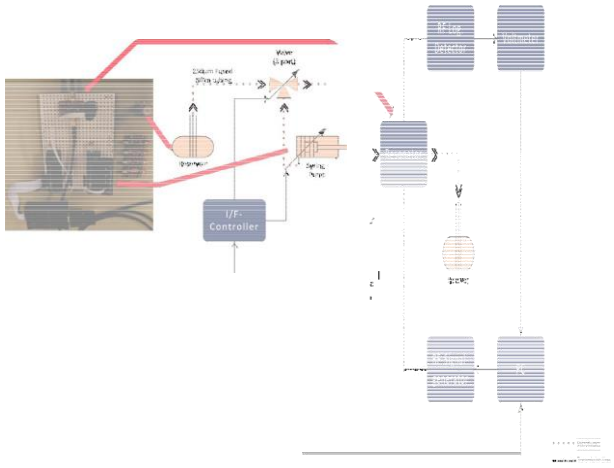

Fig 7. Micro fluidic Breadboard System.

V. CONCLUSION AND OUTLOOK

Research in automation of AFM-based nano manipulation is still at an early stage. Several challenges such as active drift compensation, scanner creep and hysteresis, and reliable manipulation protocols have to be addressed simultaneously. A flexible AFM control software/hardware architecture was developed that will help to develop methods for automated manipulation of nano scale objects such as CNTs, DNA etc.

Regarding the structuring of biological components of biosensors, the methods, presented in this paper, shows promising results for the structuring of biocomponents with sizes between the nm and µm range. Nevertheless, for future experiments it will become necessary to solve the cleaning of the AFM tips, to provide a reliable and industrial feasible method.

First simulations and principles for the design of a new signal acquisition unit were shown as well, to set up diagnostic systems without complex optical setups or fluorescence dyes. The currently used approach is feasible for the usage of biological cells and biomaterials. However, future work has to deal with an integration into useable application scenarios and with the integration into commercial relevant diagnostic systems. Also the stability of the measurement as well as the acquired data has to be evaluated.

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