Automated cell characterization by a nanohandling robot station

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Abstract—Current research work on the development of a nanohandling robot station for automated characterization of biological cells is presented. The station consists of a sample piezo scanning stage with three degrees of freedom (DoF) and a three-axes nanomanipulator that is equipped with a piezoresistive atomic force microscope (AFM) probe as an endeffector. Thus, the endeffector can be used both to measure mechanical properties of the sample and as a force sensor giving a feedback signal to the underlying control system. While the endeffector can be positioned coarsely by the nanomanipulator, fine positioning of the sample is performed by the piezo stage. As a visual sensor for the station an inverted optical microscope is utilized. The setup is enhanced by a second nano-manipulator equipped with a pipette for injection/extraction tasks or patch clamp purposes. Alternatively, a second piezoresistive AFM probe can be used as an endeffector for the second nanomanipulator. The station provides the opportunity to automatically determine a region-of-interest (i.e. a single cell) by means of object recognition in previously acquired images from the optical microscope. Next, the endeffector is brought into proximity to the sample, and an automated, mechanical characterization of the cell is performed. First experiments cover recordings of single force-distance curves to gain information on elasticity and adhesion of the cell. Using the fast piezo stage, complete adhesion or elasticity maps of the region-of-interest can be obtained. This mechanical characterization delivers valuable information about cell mechanics concerning cell to cell contact or cell motility. It is also of interest in oncology, since tumor cells tend to have a different elasticity than healthy cells. The use of a second nanomanipulator surpasses the capabilities of a standard AFM system, since it enables the station to cover areas as deoxyribonucleic acid (DNA) extraction, measurements of reactions to drug injection or electrophysiological measurements of stress activated ion channels.

I. INTRODUCTION

Characterization and manipulation of objects on the nanometer scale play an important role in the context of nanotechnology, material research and biomedicine. While scanning electron microscopy (SEM) is applicable for most research domains, its need for the sample to be placed inside a vacuum chamber makes it unsuitable for cytological and other biological studies with living objects.

In comparison to the SEM, the AFM has been proven to be a valuable instrument for characterizations of biological objects because of its ability to work under versatile conditions (i.e. in aqueous environments) and its high spatial resolution. Since the tip of an AFM probe can be brought into direct contact with the sample, an AFM can also be exploited for nanomanipulation tasks, like cutting structures or applying stress [1]. Thus, atomic force microscopy has become an attractive tool for imaging as well as manipulating biological samples at the cellular and even sub-cellular level [2], [3].

Besides imaging and manipulation, another field of application of AFM based techniques is the molecular recognition force microscopy (MRFM) that enables the AFM to detect complementary molecules and measure affecting binding forces. Here, the probe tip is functionalized by the attachment of molecules (e.g. antibodies, ligands) which bind to analytes (e.g. antigens, receptors) on the sample (described in detail in [4]). Results of such experiments are useful for pharmacological studies, e.g. where activation processes of immune cells are analyzed [5]. Combinations of MRFM with single molecular functional studies, like patch-clamp on individual ion channels, will lead to a more, detailed knowledge of biological processes [6].

Functionalized cantilevers can also be utilized to measure forces between cells. E.g. adhesion forces can be measured, which is of primary importance for multicellular organisms, neuronal path finding, and binding of leucocytes to infected cells [7].

Mechanical characterization of cells is of interest when analyzing cell motility or the structure of multicellular tissues [8]. Many age-related progressive diseases are...
expected to derive from changes of the elasticity of cells, caused by changes in the cytoskeleton [9]. For studying membrane stiffness and the elasticity of epithelial tissue, non-functionalized cantilevers are commonly used.

Studies of some E. Coli bacteria strains form another challenging research domain in which AFM techniques are used [10]. Here, especially the structure and adhesion of the bacteria’s fimbriaes are of particular interest, because they act as an instrument to transfer DNA and thus drug resistances from one bacteria to another.

Due to the short degradation time of the bacteria, the cooperation of high resolution imaging, nanomanipulation and recording of force measurements has to be performed within a very tight time frame. Thus, such experiments have to be executed in a highly automated manner, which is implemented in the automated nanohandling robot station presented in this paper.

II. EXPERIMENTAL SETUP

First measurements have been conducted with a preliminary setup, which is depicted by Figure 1. The 3-DoF piezo scanning stage (PIHera, Physik Instrumente, Germany), with an attached holder for a glass slide or Petri dish containing the biological sample to be analyzed, is mounted on the specimen stage of an Axiovert 200m inverted optical microscope (Zeiss, Germany). The integrated capacitive sensors allow the piezo stage to be operated in closed-loop mode.

A three-axes nanomanipulator MM3A (Kleindiek, Germany) is attached on the base of the scanning stage. It offers a resolution of 5 nm in x- and y-direction and 0.5 nm in z-direction. As an endeffector for the nanomanipulator, piezoresistive cantilevers provided by NaScuTec (Nanoscale Technologies, Germany) have been used. These AFM probes have a pyramidal shaped tip with a radius below 10 nm and a height above 17 µm.

As the goal is to perform automated measurements and to use further tools in addition to the piezo-electric cantilever, a setup consisting of more powerful and flexible components has been chosen for future measurements. This setup includes a different piezo scanning stage (PIMars P-563, Physik Instrumente, Germany). The closed loop travel range of this nanopositioner is 300 × 300 × 300 µm, with a resolution of 1 nm and a step and settle time of 15 ms, which is twice as fast as the previously used stage. The stage is equipped with an aperture, placing the sample in the middle of it, instead of next to it.

According to the spatial reduction and the added weight of tools different from cantilever, a system of linear axis will be used for coarse positioning of the tools. Their travel range is 7 mm, with a step width of 50 nm and the capability to lift up to 10 N (SLX 0715, SmarAct, Germany). Another advantage of the linear axis is that the angle of the cantilever to the specimen and substrate can be chosen more precisely, since changing of the height does not influence this angle as with the non-cartesian system of the MM3A.

Additionally, the resulting setup became much more compact, which minimizes possible vibrations due to long tool arms or sample holders.

III. CONTROL SYSTEM

Figure 2 shows the control scheme used for the experiments described below. The mechanical characterization of biological cells is performed by recording of force-distance curves that are gained via pushing of the sample against the piezoresistive AFM probe. A bending of the cantilever results in varying resistance that is converted to a detectable voltage by a four-wire Wheatstone bridge. This signal is amplified and digitized by a custom-made bridge amplifier, and transferred to the control PC via the universal serial bus.

A. Control System Architecture

To keep the control system as flexible as possible and to allow a simple access to different control and sensor modules, a client/server architecture with communication over TCP/IP is implemented.

On the sensor side, there is an image processing unit that serves for acquiring images from the microscope, recognizing objects (endeffector and regions-of-interest on the sample), and tracking their position. Additionally, the voltage that is measured at the Wheatstone bridge of the cantilever and that represents cantilever’s deflection, is processed and sent - with the other sensor data - to a sensor server. These data are made available to any client who requests it.

A low level control module is responsible for the access to the actuation hardware and for the execution of task primitives. While executing such a task primitive, sensor data gained by the sensor server can be used as feedback data. A common task primitive is, for example, the positioning of the endeffector by using the image processing data as feedback. A high level control server sends control commands to the different low level control units. The selecting a region-of-interest, the sending of commands for starting or stopping the tracking of objects, and the setting of various parameters of the image processing belong to the responsibilities of the high level control module. For the low level control units it can be determined whether the execution of the control primitives and may determine some of the low level control units to acquire...
data from a teleoperation device if manual operation is selected. For semi- or fully automated tasks this high level control server acts as an interface allowing the GUI to initiate the execution of these complex responsibilities. By monitoring all necessary sensor data, it is also able to stop the execution of the lower level tasks in case of unpredicted behavior.

Due to this modular architectural design, which has been previously developed by the division, exchange of the user interface as well as the hardware components can be implemented without difficulty. A schematic model of the architecture is depicted in Figure 3.

B. Manual Control

Even if the station is designed for an automated characterization of the sample, the user has the opportunity to perform teleoperated experiments. Firstly, the nanomanipulator can be positioned manually, either by an input device or the graphical user interface (GUI), while the image acquired from the optical microscope is giving the user visual feedback. Additionally to the height information gained from the focus scan of the microscope, the sensor data coming from the piezoresistive cantilever are used as a safety mechanism to avoid tip crashes. Secondly, the piezo scanning stage holding the sample can be positioned manually to a desired location. Force data produced by the piezoresistive cantilever are used to limit the movement of the piezo stage to avoid an increase of the cantilever load over a previously selected threshold. This reduces the risk of damaging the cantilever tip and sample. Due to the integrated capacitive sensors, the stage can be operated in closed-loop mode.

As an auxiliary input method, a haptic interface can be used to move the scanning stage and the sample, respectively. Here, the affecting forces on the AFM cantilever are rendered on the haptic device to give the user additional feedback of his manual operation. This allows the operator to accomplish tasks like applying stress to the cell in a more realistic way, because he can feel the scaled applied force while pushing the sample against the AFM probe.

C. Automated Control

To accomplish highly automated measurements, a complex control system has to be developed. This system has to cover object recognition and tracking tasks by analysis of images gained by the inverted optical microscope. As an additional sensor, the difference voltage at the Wheatstone bridge of the piezoresistive cantilever is measured, representing the actual bending of the cantilever.

The use of an optical microscope as a visual sensor to recognize the three-dimensional position of endeffectors and biological objects has already been realized by a previously developed cell handling station [11]. The coordinate system used in the experiments is defined as the microscope stage being the x-y-plane and the z-coordinate being normal to that plane and parallel to the movement of the microscope’s focus drive.

To avoid damage to the AFM tip, the endeffector has to be first placed at a height reasonably far above the focus plane of the sample. Initially, neither the endeffector nor the sample is entirely in focus and therefore, the geometric model finder algorithm used for object recognition is unable to detect both objects. To deal with this situation, the control system first has to perform a focus scan throughout the full focus range by moving the microscope’s focus drive with constant velocity. Images are grabbed in defined intervals and the focus index algorithm from the Matrox Imaging Library\(^1\) is applied to detect and correlate edges in the microscope image.

This procedure yields to two distinct focus peaks, one representing the plane of the sample and the other the z-position of the endeffector. Since the sample plane does not move during an experiment, it can be assumed to be fixed for the duration of the experiment.

For an automated approach of the endeffector towards the sample, it is necessary to measure the endeffector’s z-position since the nanomanipulator (both MM3A and the SLX 0715 system of the planned system) is not equipped with sensors to measure its own displacement. To measure the z-position, the microscope’s focus will be used. Since the two distinct focus peaks merge into one broader peak while moving the endeffector into proximity of the sample, a reliable detection of the z-position only by the focus index algorithm is difficult. Instead, after the endeffector’s initial z-position is detected, a geometrical model finder algorithm is used to measure the similarity of a predefined model and the endeffector. The value representing this similarity is also a good estimator for the focus quality of the object detected by the algorithm. This information can be exploited to implement a closed-loop control for the microscope’s focus drive, allowing the image to be continuously and exclusively focused on the manipulator’s endeffector. This procedure is described in detail in [12] and has been already developed by the division. It will be part of the future setup.

The task of approaching the endeffector towards the sample will be subdivided into two parts: First, the scanning stage is moved to its lowest position and the automated focus scan is executed. The endeffector is then moved along the z-axis with relatively high velocity while the implemented autofocus is following the previously detected shape of the cantilever. When the focus plane reaches the threshold defined by the sample plane plus

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\(^1\)http://www.matrox.com/imaging/products/mil/home.cfm
half of the vertical scanning range of the piezo stage, this coarse approach will be stopped. To achieve a fast coarse approach without the risk of damaging the tip of the cantilever, the vertical velocity could be selected depending on to the distance between the endeffector and the sample plane.

Second, due to its better resolution, the piezo stage carrying the sample will be slowly moved towards the endeffector until a slight bending of the piezoresistive cantilever is detectable by a varying voltage at the Wheatstone bridge. After retracting the piezo stage by a small distance, force distance curves can be acquired.

The automation of the tip-sample-approach enables the station to record not only single force distance curves, but even perform such measurements on a grid covering the previously selected region-of-interest in an automated manner. The recording of force distance curves is performed by movement of the piezo stage. Due to its integrated capacitive sensors and the provided piezo controller, accurate actuation of the stage is feasible.

To avoid tip crashes, the microscope image may also be used as a safety mechanism. Previous experiments have shown that the reflective surface of the cantilever produce a significant bright dot in the microscope image if the cantilever is bent heavily. This may be exploited as an additional safety mechanism.

It is planned to enhance the system by a digital signal processor (DSP) for controlling the piezo stage. The Signal Ranger SR-SP2 (softDB) fixed point DSP board is selected, because it is reasonable cheap and provides eight analog input and output connectors which can be easily connected to the piezo controller. The already mentioned custom made bridge amplifier has to be modified to allow the analog signal to be transferred directly to the DSP board. This will facilitate the implementation of a closed-loop PID controller holding the deflection of the cantilever at a constant value in real-time and allowing fast movements in the x-y-plane without lifting the endeffector off the sample. If the resolution of the piezoresistive cantilevers turns out to be sufficient, even AFM scanning operations could be possible.

IV. CHARACTERIZATION OF BIOLOGICAL CELLS

A. Calculation of the Elastic Modulus

First experiments for the characterizing biological cells are focused on mechanical characterization. The setup is designed for measuring the elastic modulus of the specimen.

Before the measurements can be conducted, the cantilever has to be calibrated. This calibration is performed on a hard substrate, according to [13], and thus is not influenced by the soft specimen. For measurements of the specimen, special care has to be taken due to indentation owing to the softness of the material, leading to force-distance curves differing considerably from those taken on a hard substrate (Fig. 4), as the measured deflection of the cantilever is superposed with the indentation into the material.

Therefore the equation describing the loading force of the cantilever has to be adjusted by a factor for the indentation

\[ F = k \cdot d(z) = k \cdot (z - \delta). \]  

Where \( F \) is loading Force, \( k \) Cantilever spring constant, \( d(z) \) cantilever deflection, \( z \) piezo position, \( \delta \) indentation depth.

Normal cantilevers with conical tips are used for this experiments, thus for describing the indentation of a conical object the Hertz model for indentation modified by Sneddon [14], [15] is used

\[ F(z) = \frac{2}{\pi} \cdot \tan(\alpha) \cdot \frac{E}{1 - \nu^2} \delta^2. \]  

Where \( F \) is loading force, \( E \) elastic modulus, \( \nu \) Poisson ratio, \( \alpha \) half opening angle of conical tip, \( \delta \) indentation depth. These two equations can be used to gain an equation for the elastic modulus \( E \):

\[ E = \frac{\pi \cdot k \cdot d(z) \cdot (1 - \nu^2)}{2 \cdot \tan(\alpha) \cdot (z - d(z))^2}. \]  

This with equation, for every force distance curve taken, the elastic modulus can be calculated. With a set of evenly distributed measurements an elasticity map of the specimen can be produced this way.

B. Measurements

For first experiments, measurements were conducted in ambient air. Air-dried adherent dead tumor cells had been chosen as specimen, which are spread on the bottom of a petri dish, forming a film of cells and cell debris with a varying elastic modulus (see Fig. 5)

In different areas of the sample, force distance curves have been taken. The measurements indicate changing adhesion and elasticity, which is expected of the film of dried cells and cell debris. Mean values of a succession of ten force distance curves have been taken for evaluation. Most areas show the characteristic behavior of softer material, the curves being non-linear and having a smaller slope than theoretical curves for a hard substrate (compare to Fig. 6).

![Fig. 4. Comparison of indentation curves on hard and soft substrate](image-url)
Additionally, large adhesion forces can be found, as on the retraction of the cantilever, the cantilever sticks to the sample for some time, until it finally breaks free. The elastic moduli measured seem reasonable for the gel-like biological material. These measurements with the preliminary setup are described in more detail in [16].

A still unexplained phenomenon was found with the measurements, as some successions of force distance curves showed a decrease of cantilever deflection over time (see Fig. 7).

It is still unclear if this is owing to an unstable sample holder, which could result in an increasing distance of sample and probe. Another problem could be the high indentation angle of the cantilever caused by the non-cartesian movements of the MMA3 or pollution of the cantilever, resulting in increasing stiffness or decreasing sensitivity. But as the effect was neither predictable nor very well reproducible, measurements with the more stable future setup will have show, if this phenomena still exists, excluding possible causes step by step.

With the realization of a cell elasticity measurement station, a reasonable foundation for a versatile cell characterization station has been build. First experiments can testify if needed resolution and spring constants can be achieved by the piezo-resistive cantilever.

With the future setup, providing increased stability, more flexibility concerning different tools, and an increased scanning speed, further measurements will be conducted. Starting again with mechanical characterization, at first distributed force distance curves will be conducted, being a valid testing ground for the setup. Accompanying, the influence of different angles between cantilever and substrate should be investigated in these measurements. An important next step will be the automation of the measurement process, in such a way that scanning of a region of interest is possible, to gain elasticity maps of the specimen. While the automation task concentrates on the software level, on the hardware side especially the cantilever holder has to be adapted to work in a liquid environment. New effects, as the capillary forces of liquids influencing the cantilever deflection have to be accounted for.

**V. CONCLUSION AND OUTLOOK**

The realization of a nanohandling station to measure cell elasticity, lays a reasonable foundation for a versatile cell characterization station. The results prove that the developed station can be applied for the mechanical characterization of biological cells. Using piezoresistive AFM cantilevers, forces in the range of $\mu$N up to nN can be detected, depending on the thickness and stiffness of the cantilever. The requirements for biological cell characterization are a demanding challenge in perspective to force resolution down to pN, but they are feasible as preliminary results show.

Based on these developments, more sophisticated setups are planned, including a second endeffector for measurements or manipulation, allowing for instance electrophysiological measurements of mechano-sensitive ion channels or recording of elasticity changes of the membrane due to injected drugs.

The described setup is indented for specimen visible under an inverted light microscope. For smaller specimen, the use of a commercial AFM in combination with an additional endeffector - for instance a piezoresistive
cantilever, an injection pipette or an electrical sensor - is being investigated. In this case, the coarse positioning will again be done with the help of a light microscope and the fine positioning by processing the AFM images. This implies an extension of the control system, in order to integrate the AFM as a sensor.

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REFERENCES