Interactive Sonification for exploring Single Molecule Properties with AFM based Force Spectroscopy

Jörg Martini, Thomas Hermann, Dario Anselmetti and Helge Ritter

***Abstract*— This paper presents an interactive audio-haptic human-computer interface for controlling an atomic force microscope (AFM) in force spectroscopy experiments on single molecules. The sensor data used are proportional to the force that is applied to a single molecule. These forces are measured in real-time by using the reflection of a laser beam from a cantilever. We present a system that involves (a) a visual display of the data (b) a force-feedback joystick for navigating the sample and providing a tactile feedback of the forces, and (c) an auditory display to monitor the measured data while interactively moving the sample. The sonification we have developed integrates information at various levels of detail, including *audifications* of the high-frequency cantilever movement, and an auditory stream that communicates the instantaneous deviation of forces between the approach and retract phase of the sample. The sonification design and offline-computed sonifications will be presented and discussed. We further report on our first experiences with this interactive multi-modal control interface for manipulation of individual DNA molecules.**

***Index Terms*— Interactive Sonification, Atomic Force Microscopy, audio-haptic control**

I. Introduction

Since 1986 [1] the atomic force microscope (AFM) has evolved into a standard surface examination tool to locate and inspect nanoscale objects at sub-nanometer precision with piconewton sensitivity at millisecond time resolution. In addition to important improvements in this field, the same setup was shown to be extensible also to perform measurements of magnetic domains of samples [2], calorimetrical properties of small samples [3] and force spectroscopy [4] of single molecules. Today the principle experimental setup measures the bending of a small (length: 10-300 μm, width: 4-40 μm, thickness: 80-1000 nm) cantilever, detected [5] by the shift of a laser that is reflected from the back of the cantilever onto a position sensitive detector. In force spectroscopy the bending of the cantilever is due to an elastic connection formed by the inspected molecule(s) between the tip of the cantilever and a sample surface. With an increase in the relative distance between the cantilever and the surface, the elastic connection stretches and the cantilever bends (Fig. 1) due to the fact that the molecule apply a force on the cantilever (and vice versa). Detectable forces depend on the spring constant of the cantilever and therefore cover a great range of about 10 pN up to 1 μN. The measurement of the force-distance dependency is called 'force spectroscopy'. In order to perform force spectroscopy measurements, a molecule must be bound between the cantilever and the surface.

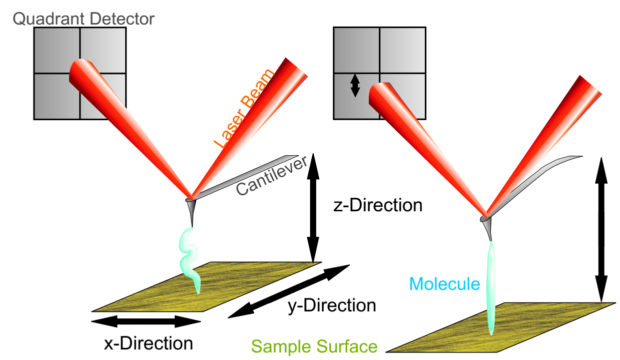

Fig. 1. Principle of force spectroscopy

In certain measurements (e.g. DNA on gold surfaces in a buffer solution) the necessary binding of the sample (i.e. DNA) between the surface and the cantilever is a stochastic and rare event. Previously a simple automatic approach/retract cycle was used to 'fish' for a molecule. At the same time, a human experimenter had to watch over the experiment, as there are numerous sources of serious or even detrimental (i.e. drift of laser-beam, floating of sample surface, drying of buffer solution, crash of cantilever etc.) mistakes that can occur during the course of an experiment. Additionally, more than one approach/retract cycle for one particular molecule is desirable, but measurement parameters (i.e. retract-distance) are unique to the molecule under examination. Therefore an interactive approach with automated subroutines for the control of the experiment has been chosen. Here we propose to improve the already existing experimental setup by using interactive sonification in such a way that information about the available data and the physical process are included in an interactive auditory loop. The sonification is designed in order to integrate information at various levels, including audification of the cantilever (which is not that different from a gramophone needle) and the force differences measured between the approaching and retreating cantilever, computed in real-time. Currently, we listen to off-line rendered sonifications which already suggest that sound can support human awareness of activities in the 'nanophysical world'. The goal of sonification is to improve the performance of the experimenter during the experimental loop, in order to obtain a higher rate of interesting experimental results. The rate of automated approach-retract cycles is approximately one binding event (not necessarily useful) in 1000 approach-retract cycles.

The paper is structured as follows: in Section II the application, experimental setup and specific problems in this domain are addressed. Section III then presents the experimental work and the tactile interface that we have developed. Benefits and drawbacks of this interface will be discussed. In Section IV we will present a sonification design that provides a real-time rendered 'soundscape' to accompany the experimenter activities. Sound examples are given and discussed in Section V. The paper closes with a discussion of the benefits of interaction in this domain and prospective future work.

II. The Application: Force Spectroscopy with AFM

A. *Force spectroscopy of a single DNA molecule*

In force spectroscopy the forces applied to a molecule bound between the tip of the cantilever and the sample surface depend, according to Hooke's law, on the bending of the cantilever and therefore on the distance between the cantilever and the surface. Certain biomolecules, e.g. DNA, show distinct features in their force-distance-curves that allow conclusions about internal structural transitions, binding partners, elastic properties etc. [6], [7]. A schematic force-distance-curve of DNA is shown in Figure 2. As the cantilever and the surface

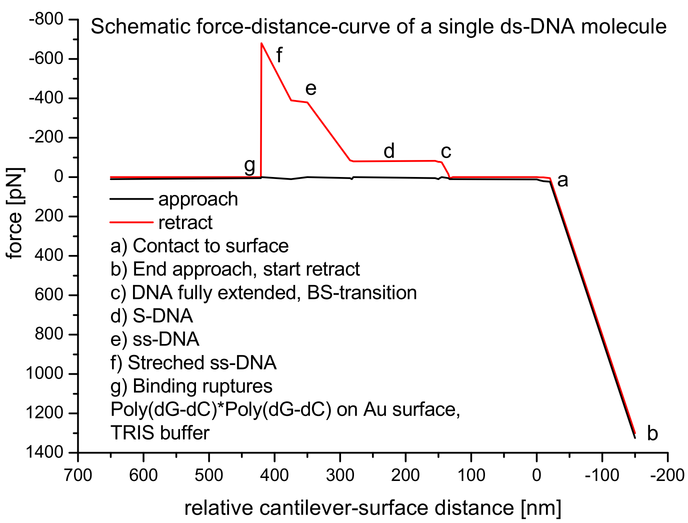

Fig. 2. Schematic force-distance-curve of Poly(dG-dC)•Poly(dG-dC) DNA

approach each other, the force on the cantilever is (close to) zero. When the cantilever comes in contact with the surface (a) a positive (arbitrary) force, that is linearly dependent on the relative distance between the cantilever and the surface, acts on the cantilever. During the retraction of the cantilever (b to a), the force acting on it is the same as during the approach, until cantilever and surface separate. Afterwards the bound B-DNA is stretched without any measurable force until fully extended (c) and then overstreched into the so called S-DNA. This is indicated by a distinct plateau (d) of about 140 nm length at a force of -80 pN. The length of the plateau is consistent with a 70% overstretching of the B-DNA and therefore varies depending on the binding-position of the DNA. A linear decrease in force to -380 pN followed by a semi-plateau (e) becomes apparent. This indicates the melting of the double-stranded-DNA, followed by the stretching of a single strand of the DNA (f) until the binding ruptures (g) at -680 pN.

In order to investigate the reversibility of the transitions (B-S-transition and melting of ds-DNA) on different time scales, it is interesting to perform multiple measurements of the same DNA-molecule at different pulling velocities. The velocity-hysteresis-dependency then provides information about the kinetic properties of the DNA-transitions. Obviously the retract movement has to be stopped before the molecule ruptures.

B. *The experimental setup*

Fig. 3 shows the experimental setup [9]. On the left side one can see the self-built AFM-head, including the piezo-table for sample-movements and an observation camera to control the laser spot on the cantilever. The right side shows the complete setup, including a sound damping cover for the AFM-head, a passive vibration damping table, crucial parts of the measurement-electronics, the observation monitor and the measurement-computer with a force-feedback-joystick (Microsoft Side Winder 2 Force Feedback).

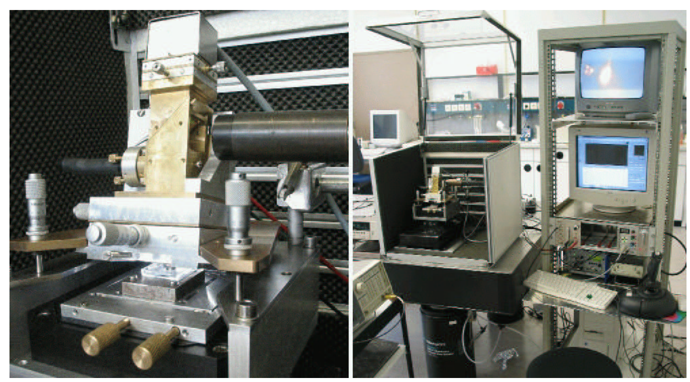

Fig. 3. Experimental setup; left: AFM head; right: complete experimental setup

The control software was written in LabVIEW (National Instruments). The control of the joystick is embedded into the measurement program by a .dll-routine.

III. The Experimental Loop

In earlier experiments thousands of force-distance-curves were obtained by repeating the approach/retract cycle with constant speed at different x-y-positions on the sample. The x-y-position was manually and more or less randomly adjusted on the controlling piezo-electronic. The spectroscopic data were analyzed after the experiment, by screening and analyzing all of the force-distance curves.

The drawback of such an repetitive control is that a human experimenter has to watch over the experiment for hours with little 'real' control. Furthermore the experiment only allowed for a single pulling of a molecule at a specific speed.

A. Interactive Control

The interactive control of the experiment that has been implemented in other groups [8], contains a force-feedback joystick for the control of the cantilever's z-position (distance to surface) and the x-y-position of the surface. The measured force is directly mapped onto the controlling z-position-axis (i.e. the vertical axis) of the force feedback joystick, quasi an amplified measurand. At the same time, the measured data (i.e. force-distance of approximately the last two minutes and the x-y-position) is displayed in two graphs on the visual display in real-time.

The z-position-control works by regulating the speed of the z-positions change. To provide a sensitive and safe positioning (because of the danger of breaking the cantilever), the z-axis value $v_z \in \{-1..1\}$ and the speed control slider value $v_g \in \{0..1\}$ define the cantilever's speed v_c according to the non-linear law [1] $v_c \propto v_z^5 \cdot v_g^2$. This way one can easily and precisely move the cantilever into the desired z-position and keep it there by moving the speed slider into the zero-position without danger of breaking the cantilever by accidentally moving the joystick's z-axis out of position.

The fire-buttons of the joystick are used to call automated subroutines such as recalibration of the zero-force-level, clearing of the display and automated data acquisition of the force-distance-dependency at different velocities. When the experimenter is aware that a molecule is attached to the cantilever and surface, i.e. when (s)he sees and feels an attractive force in the right position of the present force distance curve, measurement parameters such as pulling distance, oversampling rate and the different pulling velocities are set and the measurement is started. The joystick's POV-button (point of view) is used to navigate the cantilever's x-y-position.

B. Discussion

The interactive control has proven to be very functional. It makes the experimenter's work a lot more pleasant as it has a game character to a certain extent. It also allows measurements that are more interesting from the scientific point of view.

Working with the joystick, it became apparent that binding rates dramatically increase when the cantilever is moved in the x-y-direction while touching the surface. As the x-y-positioning is not very precise and problems with horizontal stretching of molecules arose, we are currently working on automated circle movements in x-y-direction, again by just pressing a fire-button.

The tactile response of the joystick has clear advantages for the experimenter, because when turned off, awareness of the forces acting, is much lower and therefore the risk of breaking a cantilever is much higher. However, the available forces of the joystick are on the one hand rather weak and the procedure (of approaching/retracting) is repeated so frequently (with about 0.1-1 Hz) that after time, the tactile sense becomes bored and attention decreases. This is the reason why we assume, that stimulation of another human sense - hearing - could increase the experimenter's performance. This is expected to lead to a reduced error rate for two reasons: it may reduce the frequency that the cantilever is pulled too far when a molecule is bound, and it may also allow the experimenter to hear if a molecule is bound and therefore avoid or stop premature retractions.

[1] any odd exponent is suitable, larger exponents increase the sensitivity in the low speed regions.

IV. Design of an Interactive Multi-Level Sonification

As analyzed in the previous sections, current AFM force spectroscopy suffer from the sparsity of useful state feedback to the experimenter. The success of the inclusion of haptic feedback to improve the experimental performance suggests to also exploit further sensory channels in order to aid the experimenter. In view of the close physical analogy between the cantilever and a gramophone needle, sound and sonification appear as a particular natural and effective means to achieve this goal. Below we report on an initial implementation of such an approach. The information can roughly be categorized into three groups with respect to the the directness, resp. the semantic level of the information content. This categorization is in accordance with Kramer's taxonomy of 'analogic versus symbolic' representation introduced in [10]. The lowest level is represented by the data series of (time, position, force, x_{probe}, y_{probe}) vectors and thus the elementary physical measurements. The next level involves the creation of more complex variables that assist the interpretation. For instance, the difference of corresponding forces during approach and retract phases $F_a(z) - F_r(z)$ represents the net acting force. Such variables are interesting candidates for integration into the auditory display. A higher level is that of 'physical interpretation' of data, meaning events (patterns in the data) for which the semantic value is already known.

A. Low-Level Information

Basically, the cantilever can be regarded as a gramophone needle. For that reason it is obvious that the human ear may already be very suited to process the instantaneous movement of the cantilever as an audible sound. The cantilevers currently used show resonance frequencies between 500 Hz and 4 kHz in liquid and thus are already in the audible range. The sonification techniques used to represent information at this 'low-level' is *audification*, which means to use the data series as instantaneous sound pressure level samples [11]. Audification is adopted as the first auditory stream in the display. The data preprocessing yields filtered data with a cut-off frequency of around 4 kHz, which well matches the highest sensitivity of humans auditory system. We found that different speed-ups can be interesting for audifications and therefore allow the speed-up factor to be controlled manually. As audible in the sound examples in Section V, the sound allows the experimenter to distinguish whether the cantilever is in contact with the surface or not. When the cantilever is in contact with the surface, a damping of the overall volume and particularly of the high-frequency content is apparent. Due to the high sensitivity of the cantilever to external influences, even environmental events like the slamming of the laboratory door are detectable through the audification.

B. Mid-Level Information

The most important information for controlling the experiment, however, is contained in the approach/retract-force curves as shown in Figure 2. The difference $\Delta F = F_r(z) - F_a(z)$ between the forces during the retract and approach phases determines net binding force, and sudden changes in the force difference correspond to the breaking of a binding between a molecule and the cantilever. For that reason, any discontinuity, resp. the derivation of the force, is an interesting variable to be used for auditory activity. The temporal evolution of the vector $(\Delta F, z)$ is thus used to control an auditory stream in real-time. Both the actual value of ΔF and its change during the interaction are of interest. For further processing we compute a discrete approximation of

$$\left.\frac{\partial \Delta F}{\partial t}\right|_{t=nT} \approx \Delta F[n] - \Delta F[n-1], \qquad (1)$$

A Model-Based Sonification [12] approach is used to generate interaction sounds that support the experimenter's perception of the binding state in an ergonomic way. A sonification model provides the link between the data to be sonified and the sound by a simulated 'virtual process'. Such a mediator is useful since most environmental everyday sounds are a consequence of ongoing physical processes, and by using a similar mechanism to relate data to sound allows to address human listening skills in a more natural way. E.g. all acoustic systems fade into a state of equilibrium without external excitation, a concept that we are well acquainted with through everyday interactions, consequently the model to be presented next implements a similar behavior.

In our application, the sonification model is an exciter/resonator. Think of the vocal system: the lung and vocal folds provide the excitation and the mouth the resonator. Pressure difference and resonance volume determine the sound in a complex way. The sound stops when the pressure difference between the inside and outside is zero. In our model, the instantaneous change of the net binding force ΔF is used to increase the excitatory source of the virtual acoustic system.

Without any interaction, constant engery loss causes the sound level to decay until the average excitation (from random variations of ΔF) equal the loss from acoustic radiation. At the same time, the absolute magnitude of the force difference ΔF, controls the resonance frequency, so that higher binding forces are audible as a higher-pitched sound, again in line with our acoustic experience that materials under higher stress resonate at a higher pitch.

As a result, sudden changes in the force will be accompanied by a sudden increase of sound level, and a change in pitch indicating the direction of change.

This level also includes information about expected distance from the surface, lateral velocity of the cantilever during navigation, etc., which were left out for the first display prototype.

C. High-Level Information

We denote as high-level semantic information the kind of knowledge that is expressed at the task level, e.g. certain patterns in the force curve in the context of a specific history can have a meaning like 'the cantilever is now in contact with the surface', or 'the binding to the cantilever has been broken'. Such explicit events can be communicated via the auditory display by integrating acoustic markers, whose salience is driven by both the significance and novelty (resp. interest of the event). While some of these events can also be operationalized by using simple rule-based decisions, patterns can be very subtle structures hidden within the data, and classifiers and machine learning techniques may be applied for the online-computation of suitable triggers for high-level markers.

To exemplify these kinds of markers, one of them, the breaking of a binding, is implemented here. As a second marker we plan to implement the expectation of a molecule binding event to the cantilever. Both of these are specific patterns in the force-position curve that can easily be extracted from the raw data.

D. Synthesis and Control

For real-time synthesis, we currently use the software package Csound in real-time mode. The audifications are computed in Neo/NST, which is a graphical programming environment that is also useful for programming the user interface and controlling the data analysis (selection, navigation). Currently we are experimenting with interactive off-line rendering, which means that the experimental data are pre-recorded for sequences of approach and retract phases and imported in Neo from an ASCII-file, so that the user currently interacts with the data by using a slider control. This allows us to optimize the sonification for later experimental use without needing the AFM in the loop.

- **Synthesis of Audifications**
  For interactive audification, the data series is written to a wave file which is then used within a granular synthesizer module running on Pd. Different from real-time mode, the user then listens to repeated small signal windows, but this is acceptable at the test stage of the sonification system. The pd patch runs on a different machine and communication is done via Open Sound Control.
- **Synthesis of Linear Resonator Model Sounds**
  The linear resonator model can be implemented as any oscillator that is fed into a set of two-pole IIR filters. Practically, the Csound *reson* operator is used for filtering, making it possible to determine the 'formants' of the system. The controls are (a) the excitation, which is modeled by using a leaky integrator using the leak rate as amplitude for the excitation, (b) the oscillator whose characteristic is determined by a table and the phase increment, and (c) the filter frequencies and gain, that correspond to the model setup. Currently, the oscillator table is randomly filled, later this can be replaced by using a high-pass filtered window of recorded data. In addition to these stream, we currently superimpose an auditory stream of rhythmically played pitched tones according to the computed net binding force. This pitch can be regarded as an auditory indicator for a molecule binding at the cantilever.

- **Synthesis of Marker/Event Sounds**
  To start with, we use a simple playback of pre-recorded sound samples, that can either contain verbal messages like ’touch’, ’break’ or ’tied’, or non-verbal marker sounds. Our expectation is that in practical use, the non-verbal sounds are superior, since they do not distract the user. Furthermore they allow the experimenter to use the manipulation of playback parameters to encode details of the detection process.

V. Sound Examples and Discussion

According to our experience, it is difficult to evaluate the new technique on the basis of sound samples alone, because the complete interactive operation is crucial. However, the sound samples give an impression of how the sound can support the interactive data analysis.

All sound examples are provided as mp3-files on our website [13]. Listen to the 32 audifications (S1-S10) for four different types of measurements first, namely: (a) data files where DNA actually binds to the cantilever, (b) data files where nothing happens, (c) data files where some unknown things happen, and (d) noisy data. The approach-retract phase plays continuously. One notices a sound change in the middle of the files. This is caused by a contact between the cantilever and the surface (see fig. 2, a to b) which effectively damps the high frequencies. The main acoustic pattern is of course the noise. The breaks are perceived as clicking sounds. Even small clicks are audible.

The next set of sound samples are interactions with the system in real-time mode, where the cantilever is manually retracted until a binding breaks. This is an atypical case because usually when a binding is detected the standard action is to stop the interactive mode and start the automatic recording, since only these data can be used for later analysis. Suitable psychophysical experiment to assess the use of sonification quantitatively would be to ask users to press a button when they are sure that something binds to the cantilever, and compare the results under various presentation types (auditory, visual, both).

VI. Conclusion

We have presented a new application for sonification, that is expected to increase human efficiency in performing an AFM experiment. As an extension of our AFM techniques to monitor the interesting data in form of graphs and plots, our modified system integrates a force-feedback joystick to navigate the cantilever and to represent the forces on the cantilever via force feedback. Our approach to augment this real-time system by using sonification has been presented and exemplified by sound samples. The auditory display combines various information of varying complexity (resp. semantic value) into a set of parallel audio streams. The design was explained and motivated by analyzing the necessary or missing information. The ultimate goal is to perform a topography measurement before starting the pulling experiments. This way one would only search in the regions of the sample surface where molecules are present. In these areas the experimenter would move the surface in x-y-direction with the cantilever right above it. From the audification data he/she could hear if a molecule is bound to the cantilever and start the measurement when this is the case. Our experience with the sonifications lead us to expect that sound may be useful for increasing the experimental efficiency. For quantitative results, however, it is necessary to conduct psychophysical experiments.

Our next steps will be to integrate and test the developed auditory display in the application. Beyond the described experiment, we see good chances to adapt the system to other experiment types, like e.g. for controlling optical tweezers.

Acknowledgement

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