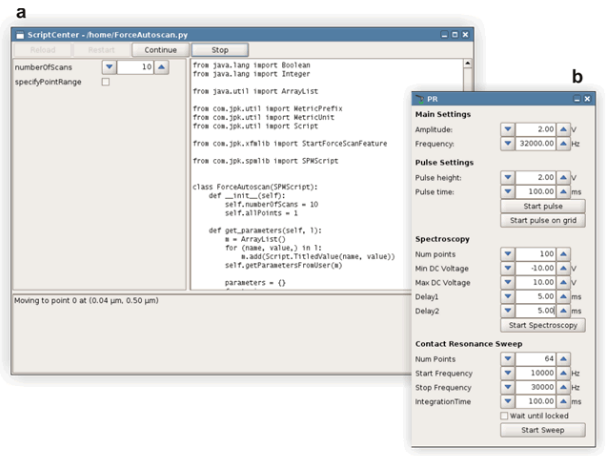
Application Note

Scripting capabilities for user defined experiments with the NanoWizard® AFM

Introduction

Commercial atomic force microscopes (AFM) systems, such as the JPK NanoWizard® come with control software which covers a variety of applications ranging from basic imaging in the common operation modes to sophisticated and complex force spectroscopy experiments. However, in cutting-edge research novel experimental protocols including accurate timing of operation tasks of the AFM are essential for obtaining the desired information. Often such research is performed using home-built instrumentation, where the researcher has full control over hard- and software including all the advantages and disadvantages of such a setup. Often the implementation of novel experimental protocols is very time consuming even though at the end of development it is just a few lines in the source code of the control software. Therefore, such experiments were mainly restricted to researchers who have a deep understanding of AFM technology including all hardware and software issues that need to be solved.



numberOfScans 10

specifyPointRange ☐

```
from java.lang import Boolean
from java.lang import Integer

from java.util import ArrayList

from com.jpk.util import MetricPrefix
from com.jpk.util import MetricUnit
from com.jpk.util import Script

from com.jpk.xfmlib import StartForceScanFeature

from com.jpk.spmlib import SPMScript


class ForceAutoscan(SPMScript):
    def __init__(self):
        self.numberOfScans = 10
        self.allPoints = 1

    def get_parameters(self, l):
        m = ArrayList()
        for (name, value,) in l:
            m.add(Script.TitledValue(name, value))
        self.getParametersFromUser(m)

        parameters = {}
```

Moving to point 0 at (0.04 µm, 0.50 µm)

Main Settings

Amplitude: 2.00 V

Frequency: 32000.00 Hz

Pulse Settings

Pulse height: 2.00 V

Pulse time: 100.00 ms

Spectroscopy

Num points 100

Min DC Voltage -10.00 V

Max DC Voltage 10.00 V

Delay1 5.00 ms

Delay2 5.00 ms

Contact Resonance Sweep

Num Points 64

Start Frequency 10000 Hz

Stop Frequency 30000 Hz

IntegrationTime 100.00 ms

☐ Wait until locked

The key to avoiding time-consuming programming of the source code is the open programming interface available in the JPK control-software which can be accessed using the modern, object oriented scripting language Jython. Since the JPK control-software is written in Java™ (Sun Microsystems, Inc.), all Java™ classes which are part of the software can be accessed by a Jython script. The functionality of such a script ranges from executing operations also accessible through the graphical user interface (GUI), applying your special altered set of parameters to direct, low-level access to the digital signal processor (DSP) or the Power PC (PPC) used for the real time control of the AFM hardware.

Example 1: Control of a syringe pump with JPK BioCell™

Forming a three-dimensional tissue from cultured cells is a hot topic in the field of tissue-engineering and regenerative medicine. Recently, Eiraku *et al.* reported self-organizing optic-cup morphogenesis in artificial culture [1]. They demonstrated qualitative comparison of rigidity by using the JPK NanoWizard® AFM with a CellHesion® module between various tissues which are distinguishable by fluorescent labeling. It clearly revealed that local phenomena such as cell growth and changes in mechanical properties are important for the formation of 3D tissue culture

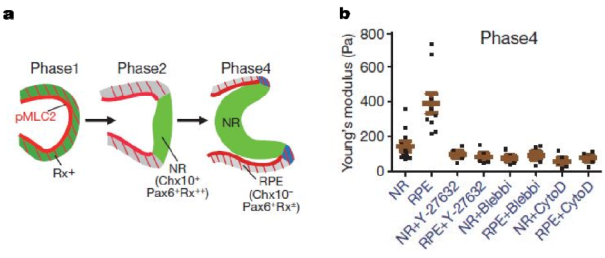


Figure 1: Schematic summary of morphogenesis of eye-cup (a). AFM-based measurement of relative tissue rigidity of phase 4 neural retina and RPE tissues with/without Y-27632, blebbistatin and cytochalasin D treatments.(b) [1]

Similar experiments for living cells and tissue can be automated on the NanoWizard® AFM with the BioCell™ and syringe pumps from KD Scientific or WPI. Figure 2b illustrates that once cells get growth factor on their surface, it triggers progression of cell cycle. In order to measure the effect of a growth factor on mechanical properties, it is necessary to control timing of injection of substances as well as the environment for living cells. Scripting capability of JPK NanoWizard® AFM enables us to control commercially available syringe pumps in ease. Since users can use all Java classes provided by JPK, this sequential protocol would be a small Jython script, which just calls several methods of JPK Java class to set parameters and trigger each measurements.

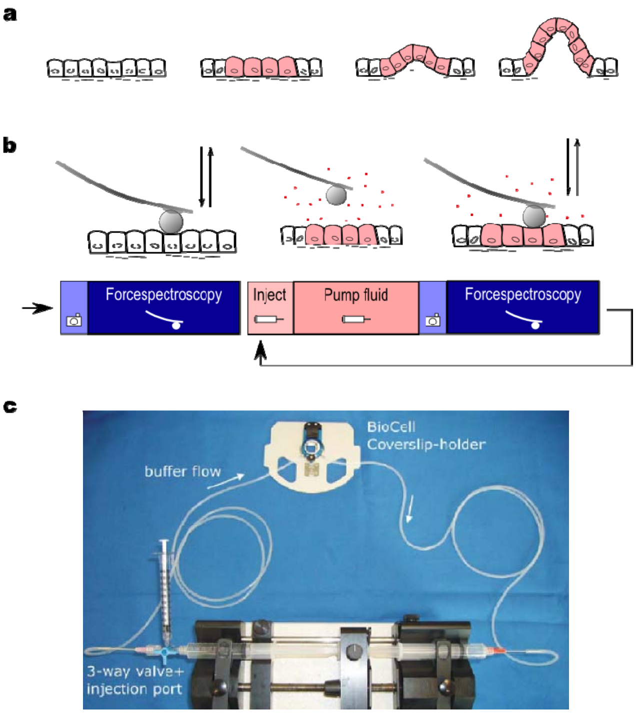


Figure 2: A model demonstrating that cell proliferation leads to formation of three dimensional tissue [2] (a)

Workflow of measurements of cell mechanical properties by a modified AFM tip (b)

BioCell™ liquid cell provides temperature control. On the picture inlet and outlet tubes are connected to the syringe pump (c)

Example 2: Extended force spectroscopy on gecko attachment system

Animals that cling to walls and walk on ceilings owe this ability to micro- and nanoscale attachment elements on their feet. The gecko, in particular its attachment system, which is the most elaborate in nature so far, is of great scientific interest. The ventral side of its toes are covered with lamellae, arrays of 3 to 5 µm thick setae divided into 100 to 1000 individual spatulae of 200 nm dimensions at the end. The spatula itself is responsible for the geckos feet adhesion to the wall.

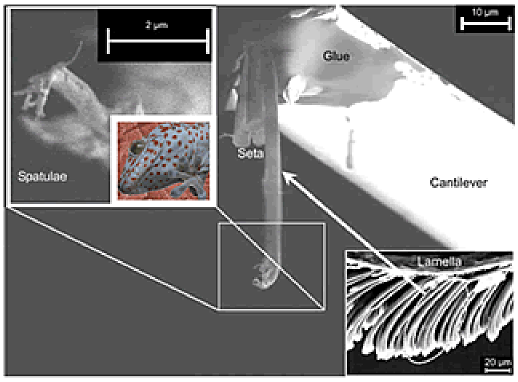


Figure 3: Electron micrograph of a gecko seta glued on the end of an AFM cantilever [2].

AFM force spectroscopy has high force resolution down to the pico-Newton scale and was applied to measure the adhesion force of the setae with high accuracy [3]. Therefore, a number of setae were glued to the end of an AFM-cantilever, extending the actual AFM-tip by length of the spatulae. Using a focused ion beam system (FIB), a single seta was prepared by cutting off the redundant setae of the attached bunch. Force spectroscopy experiments were carried out with these modified probes. An essential step in the measurement of the adhesion force is to mimic the gecko's toe's uncurling motion which aligns the spatula in contact with the substrate.

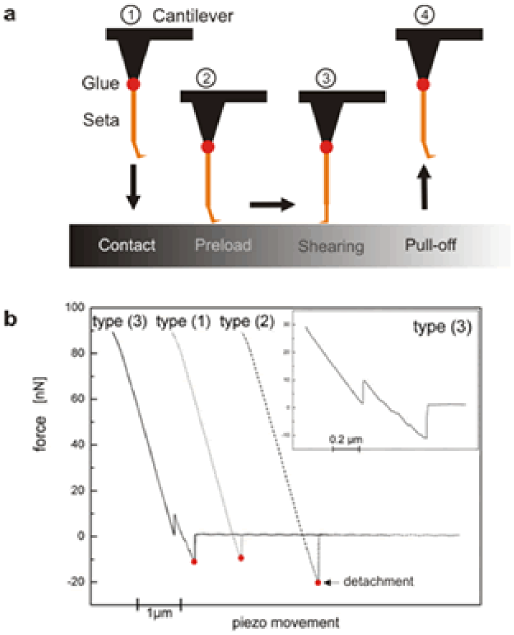


Figure 4: Modified force spectroscopy protocol for measuring the adhesion between the gecko seta and surface (a). An additional alignment step (shearing) is performed using the scripting capabilities of the JPK AFM software. Typical detachment-curves of single spatulae are shown in (b).

Example 3: Coupling of instrumentation

Advanced experiments often require external equipment to be synchronized with the experimental protocol of the AFM. Modern measurement instrumentation often provides an interface (such as Ethernet, RS232, GPIB or USB) for remote control and data exchange which easily can be accessed by a Jython script.

One example is the combination of JPK NanoWizard® II with a Raman spectrometer used in tip-enhanced Raman scattering (TERS) experiments. TERS is a technique that combines the established method of surface-enhanced Raman scattering (SERS) and scanning probe technology to deliver chemical information (vibrational spectra) and sample topography at high spatial resolution down to approx. 50 nm [4]. In the experiment, the metal coated AFM-tip used for field enhancement has to be placed into the focus of the excitation laser of the Raman setup as shown in Fig. 4a using the tip scanner of the AFM head. To record the AFM topography, the sample is scanned while the tip is fixed at the position of the excitation laser defined by the optical setup. If the sample-topography is known, TERS spectra can subsequently be taken at defined positions (e.g. a grid of positions). Fig 4c shows as an example a transmission electron micrograph and an AFM image of a tobacco mosaic virus [4] where positions 1...4 were selected in the AFM-image and the corresponding TERS spectra were taken sequentially to gain insights into the chemical structure of the virus, allowing identification of different protein and RNA species.

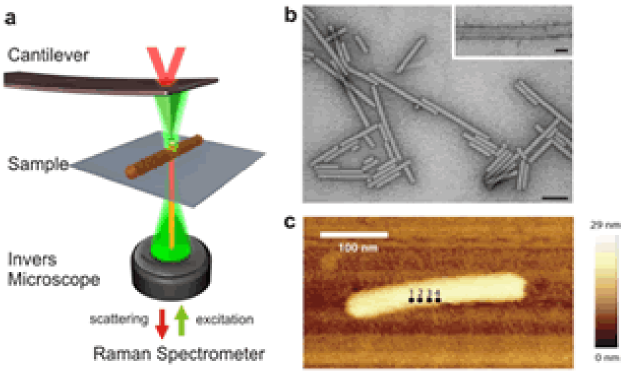


Figure 5: TERS experiments on tobacco mosaic virus: Experimental setup (a) and transmission electron micrograph of the tobacco mosaic virus (b). TERS spectra were taken on position 1...4 selected directly in the AFM topography scan (c) [4].

To automate this, the AFM software and the software of the Raman spectrometer were linked via a script on each end using a simple Ethernet connection (TCP/IP protocol) as shown in Fig. 5. The Raman spectrometer can request the AFM to approach a new position whereupon the AFM acknowledges the arrival end then records a spectrum associated with that position. The result is a Raman map, which can be overlaid on the AFM-image, so that the correlation of the topography and the Raman signal can be used to relate the chemical signal to the sample.

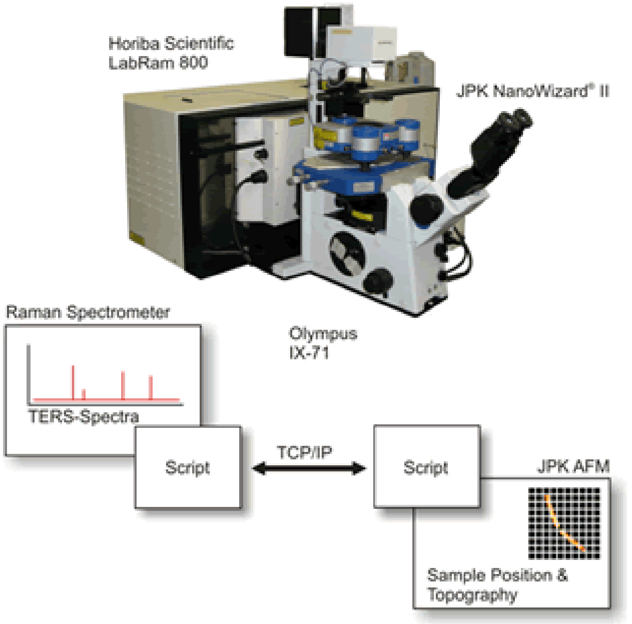


Figure 6: An example of coupling a Raman spectrometer (in this case a Horiba Scientific, LabRAM800) and JPK AFM NanoWizard® AFM via Ethernet (TCP/IP) for automated TERS mapping.

Conclusion

Users can implement unique experiments by the scripting capabilities of the NanoWizard® AFM quickly. Jython scripting is easy and convenient to modify and combine measurements even for starters. Moreover, accessibility to all Java classes enables advanced researchers to fully control NanoWizard® AFM.

References

[1] M. Eiraku, *et al. Nature* 2010, 472, 51-58

[2] S. Huang and DE. Ingber *Nat. Cell Biol*., 1999, 5, E131-138

[3] G. Huber, *Biol. Lett.* 2005, 22, 1:2-4.

[4] E. Bailo, V. Deckert, , *Chem.Soc.Rev.* 2008, 37, 921-930.

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