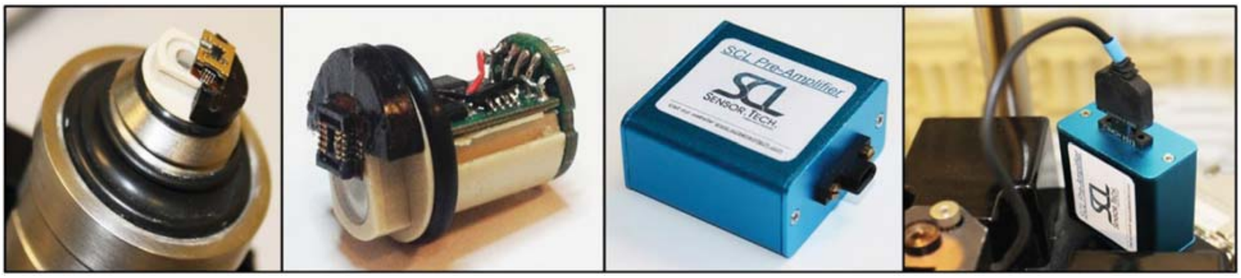
Keysight Technologies

Imaging with Self-Sensing Cantilever on Keysight 5500/5600LS Atomic Force Microscopes

Application Note



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Introduction

Atomic force microscopy (AFM), a key member of the scanning probe microscopy (SPM) family, has developed into an invaluable tool of significant importance in many different areas of research and industry. Since its first development in 1986 [1] AFM has undergone much advancement, not only to extend measurement capabilities and to increase acquisition speed, but also to make the instrument more robust and user-friendly. Most commercial AFM systems today still use optical deflection detection to image samples of interest with nanometer resolution. Whereupon easy to handle and user-friendly self-sensing cantilevers are still mainly limited to home-built systems and special applications. Therefore SCL-Sensor.Tech. Fabrication in close collaboration with Keysight developed a hardware kit, making the benefits of user-friendly and easy to handle self-sensing cantilever technology available for Keysight AFM customers. In this application note a brief technical background of self-sensing cantilevers and the developed Converter Unit (CU) is introduced. Some representative images acquired with different modes of readout and excitation are also provided. In addition, the capability of using self-sensing cantilevers to image biological samples in different media including dry state, deionized water, physiological relevant buffer solutions, as well as non-transparent liquid are also demonstrated.

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Self-Sensing Cantilevers and Instrumentation

In the last few years, excellent progress has been achieved to overcome limitations of the complex optical readout system, such as development of self-sensing cantilevers based on piezo-resistor to improve usability and handling [2]. For these cantilevers a sensing piezo-resistor is built on the silicon at the cantilever base via ion implementation. A cantilever deflection caused by a slight change in topography alters the resistance of the piezo- resistor. The variation in resistivity leads to an electrical signal which is used to generate the topography image of sample with nanometer resolution. As single piezo resistor readout cantilevers can experience problems such as thermal drift and self-heating of the piezo-resistor, the implementation of four piezo-resistors in Wheatstone bridge configuration is preferred [3, 4]. In Wheatstone bridge configuration, two of the piezo-resistors located at the cantilever are used for sensing to increase the sensitivity, while the other two resistors are located at the silicon chip. The proximity of the four resistors stabilizes the system and reduces external influences to a minimum level. In standard dynamic mode measurements, a classical acoustic excitation from a piezo actuator can be used. Additionally, one can also use thermal bimorph excitation via a heater loop on the cantilever depending on the cantilever type [5, 6]. In spite of the numerous benefits of the self-sensing technology, especially in terms of usability and the potential in automation, it has been regarded as less sensitive with high level of noise, until recent work shows that the electrical readout can at least equal optical readout in terms of noise [7].

SCL-Sensor.Tech. has established a solid self-sensing cantilever platform which serves ideally the development of cantilevers for a wide range of new applications. Currently there are many types of contact and intermittent contact mode cantilevers with different physical parameters available. The standard cantilevers are equipped with silicon tips with a nominal tip radius of less than 15 nm. Special cantilevers include tipless and diamond tip cantilevers, conductive cantilevers, and high aspect ratio Focused Electron Beam Induced Deposition (FEBID) or Focused Ion Beam (FIB) cantilevers. Tipless cantilevers are used for general deflection sensing applications, such as gas sensing [8], while diamond and FIB/FEBID cantilevers are designed for imaging special features like narrow and deep structures with highest resolution. Conductive cantilevers have been successfully applied for spreading resistance measurements (http://getec-afm.com/index.php?id=15). Different images of a self-sensing cantilever and a sketch of the implementation and its working principle are illustrated in Figure 1 (A).

The Keysight 5500/5600LS AFM system is a high-performance instrument which supports common contact and intermittent contact mode with acoustic or magnetic excitation, highly sensitive force measurement modes, and has been widely used for many different applications. It also implements sophisticated techniques such as recognition imaging (TREC) and microwave imaging (SMM). Due to the modular architecture, the 5500/5600LS is perfectly suited for self-sensing cantilever integration. In this work, a fully equipped 5500 microscope including SPMII controller, head electronic box, MAC Box, TREC Box, fixed stage with a large scan size, closed-loop scanner has been used.

A scheme for realization and implementation of the Converter Unit (CU) is given in Figure 1 (A), which illustrates the above described self-sensing cantilever concept. Using SCL self-sensing cantilevers requires a modified nose cone for carrying and connecting the self-sensing cantilever with the AFM system. The mechanical part of the nose cone is well compatible with the original Keysight 5500/5600LS nose cones, giving the possibility of positioning the cantilever using the original CCD camera system. The self-sensing cantilever, which is mounted on a printed circuit board (PCB) with a connector on the backside, is connected to its counterpart on the self-sensing cantilever nose cone (B). The self-sensing cantilever nose cone fits the Keysight 5500/5600LS scanner and uses its original pinning. The nose cone includes an instrumentation amplifier for the electrical deflection signal as well as the voltage supply for the Wheatstone bridge on the cantilever. The constant voltage supply to the self-sensing cantilever can be set at 2.048V to obtain higher sensitivity, or at 0.51V as recommended for imaging in liquid environment. An additional piezo is included in the self-sensing cantilever nose cone for driving the cantilever acoustically when used in intermittent contact mode. Furthermore, depending on the type of cantilever, a thermal excitation using the cantilever implemented heater loop and Keysight patented MAC Mode is also possible.

The second hardware component (C) is situated between the scanner and the AFM base, with connection to the photo detector connector. This component contains signal modification, amplification and the DC offset compensation. An offset compensation is necessary because of slight disparities of the piezo resistors due to fabrication tolerances. The DC offset compensation is realized via adding a certain DC value to the electrical deflection signal. The non-cantilever depending DC offset can be seen in the oscilloscope monitor in the software as well as on the head electronic box (HEB) as deflection signal at 0V setpoint. The compensation is done manually via the tip bias window in the software, therefore the “Tip Bias” has to be adjusted to get a signal around 0V in the oscilloscope monitor and at the HEB deflection display.

The self-sensing cantilever deflection signal is amplified and modified to be transmitted to the photodetector pins described in the following: The topography depending deflection signal is amplified, inverted and a DC offset is added to both the inverted and non-inverted signals. The non-inverted signal is handed over to the photodetector pins A and B, representing the upper two segments of the photodetector. Consequentially, the second signal is handed over to the photodetector pins C and D, representing the lower part of the photodetector. On one hand, the head electronic generates a sum signal from these signals, which allows approaching the surface without any optical signal; and on the other hand, it also generates a single deflection signal from the 4 signals applied to the photodetector pins A-D. This pathway is followed by both static and dynamic mode and no additional changes to the hardware or settings to the software are necessary.

The excitation signals for measuring in dynamic mode are directly collected from the low voltage connector at the AFM base. The voltage supply for the Converter Unit as well as for the self-sensing cantilever nose cone is directly from the photodetector connector.

In summary, the hardware to perform self-sensing imaging on the 5500/5600LS systems only requires the nose cone and the Converter Unit, with no need of any other external equipment or changes to the original hardware.

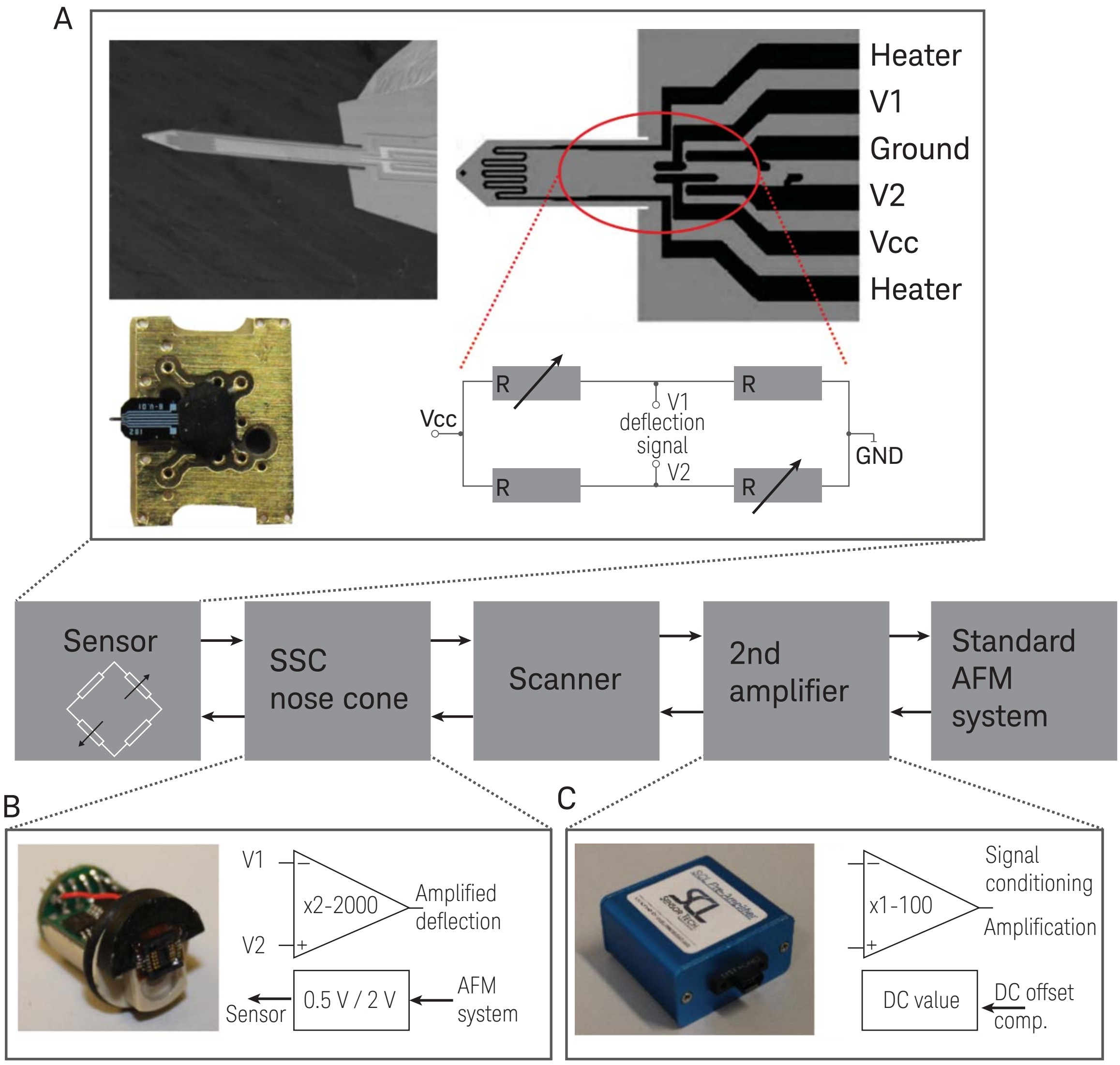

Figure 1. (A) (Top left) SEM image of a 300x100µm self-sensing cantilever including a heater loop for thermal excitation and (bottom left) an image of the cantilever bonded to the PCB board for connecting the self-sensing cantilever nose. (Right) A schematic drawing of the cantilever with a detail sketch of the implemented Wheatstone bridge. (B) The developed self-sensing cantilever nose cone. The nose cone includes the low noise voltage supply for the cantilever, an instrumentation amplifier and the piezo for the acoustic excitation. In case of thermal excitation, the heater loop is connected to the excitation system of the AFM and MAC Mode. The second hardware component placed between scanner, base and photodetector connector contains the needed signal conditioning, additional deflection amplification and the cantilever offset compensation.

The design and construction of the self-sensing cantilever CU for the 5500/5600LS Keysight AFM has been particularly focused on usability, easy plug & play implementation for the customer, and combining the benefits of the AFM system with those of the self-sensing cantilevers for performance optimization.

Proof-of-Principle Measurements

All images shown in the next session have been recorded on standard x, y calibration grating sample (TG3D-AFM) except the measurements in non-transparent liquid, where a standard line grating sample has been used (TGZ02).

For proof of principle and as a comparison with the conventional optical readout system, standard calibration gratings have been used for AFM imaging. The first set of images in Figure 2 (A-F) was obtained with different imaging modes using the self-sensing cantilever kit. A-C show dry state imaging in conventional contact mode (A), intermittent contact mode using acoustic excitation (B) and bimorph thermal excitation (C). The images D-F show the corresponding images recorded in deionized water. As the electronics embedded in the nose cone is sealed and just the cantilever itself is in contact with the liquid, no cantilever passivation is required. Here only the voltage supply for the cantilever integrated Wheatstone bridge is lowered to 0.51V for imaging in liquid. Images G-K show the corresponding optical images obtained with the same modes in both dry state and in liquid. Instead of thermal excitation, the Keysight supported MAC mode, which drives a magnetically coated cantilever in an alternating magnetic field, has also been used. In comparison, all the recorded images look similar regarding resolution, x, y, z accuracy as well as the cross section profiles. The main advantage is that there is no need for laser alignment for accurate electrical readout, resulting in enhanced usability and greatly reduced time to get the first AFM image. In case of electrical readout, instant approaching and imaging directly after mounting the cantilever is possible, both in dry state and in liquid.

A more representative application of self-sensing cantilevers is the capability of imaging in non-transparent liquids. (L) in Figure 2 shows a line grid in a petri dish filled with milk, and (M) shows the corresponding AFM topography image. A standard self-sensing cantilever without passivation in intermittent contact has been used to record this image.

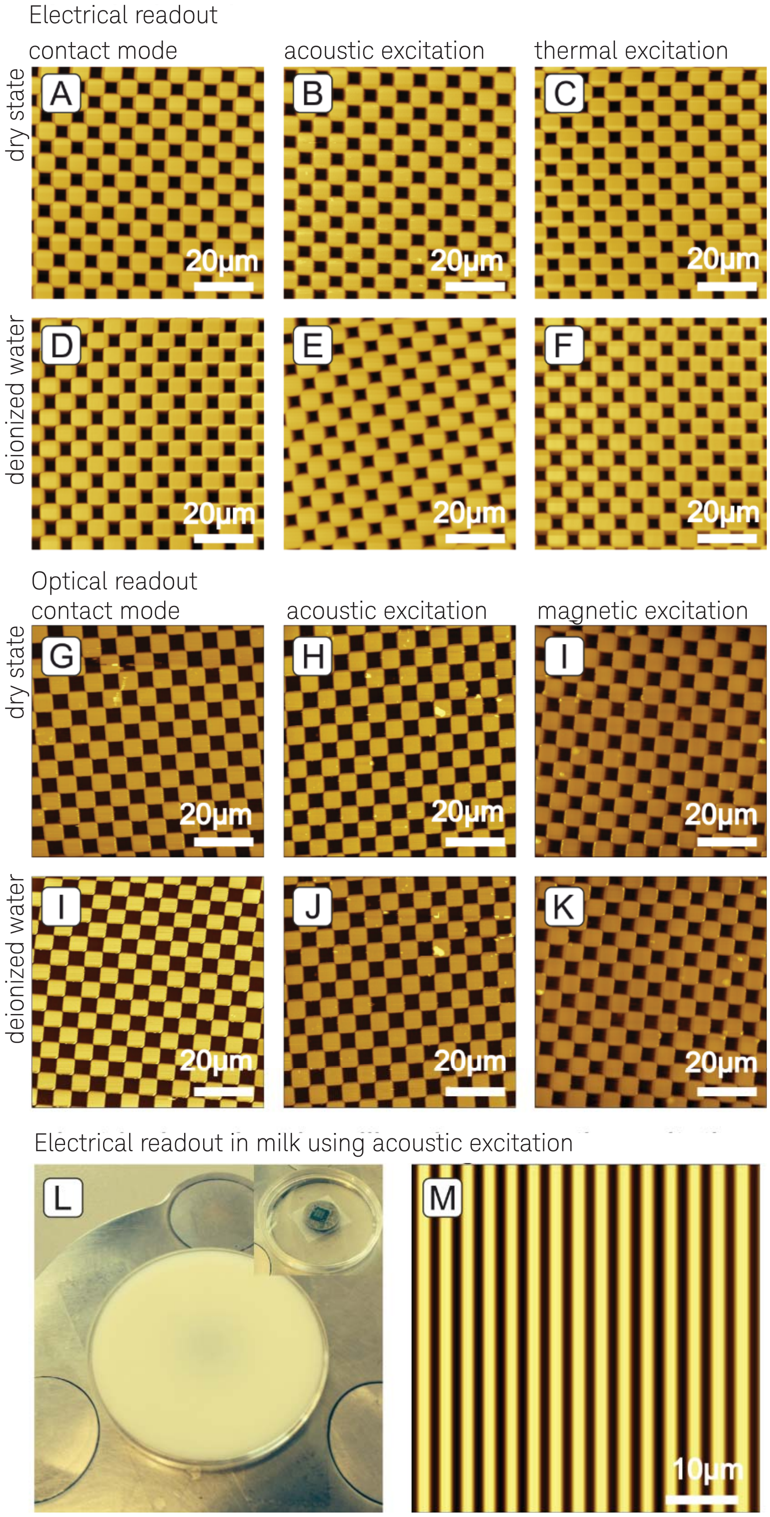

Figure 2. Topography images of calibration standard sample in different combinations of readout and excitation. (A-F) Electrical readout in contact mode, thermal and acoustical excitation in dry state (A-C) and in deionized water (D-F). (G-K) Control images recorded with a commercial cantilever and optical readout. Instead of thermal excitation, magnetic excitation is applied. (L) Line grating standard sample in milk as reference for non-transparent liquid (inset shows the same sample in deionized water). (M) Corresponding AFM image recorded in milk using a self-sensing cantilever in intermittent contact mode (acoustic excitation). Z scale: 800 nm (A-K), 200 nm (M).

Imaging of Biological Samples

For all platelet samples, freshly cleaned glass slides were incubated with a droplet of blood. After different incubation time period (1, 3 or 5 minutes) the samples were carefully rinsed with PBS buffer (140mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 1.8mM KH_2PO_4). The cell samples were gently fixed using 4% Glutaraldehyde for 15 minutes, followed by an additional rinsing step and drying with a gentle stream of nitrogen. Figure 3 (A-F) were recorded in dry state in intermittent contact mode with acoustic excitation using the self-sensing cantilevers, and with magnetic excitation using magnetically coated cantilevers. The right column represents the images recorded using the self-sensing cantilever conversion unit, while on the left side the control images of the same sample area recorded with a commercial magnetically coated Type VII MACLever is given. Figure 3G represents platelets recorded in PBS buffer after 1-minute incubation using the self-sensing cantilever hardware.

From an imaging point of view, it can be clearly seen that the self-sensing cantilever shows the same performance regarding resolution and noise at least at large scan sizes, especially when considering the huge benefit of much easier handling. Particularly for non-frequent AFM users, the average time required to get the first image is reduced dramatically due to the simplified setup. Besides, overcoming the needed equilibration time is important for time critical measurement, e.g. live cell imaging, fast changing surfaces or in situ imaging while changing the environmental conditions.

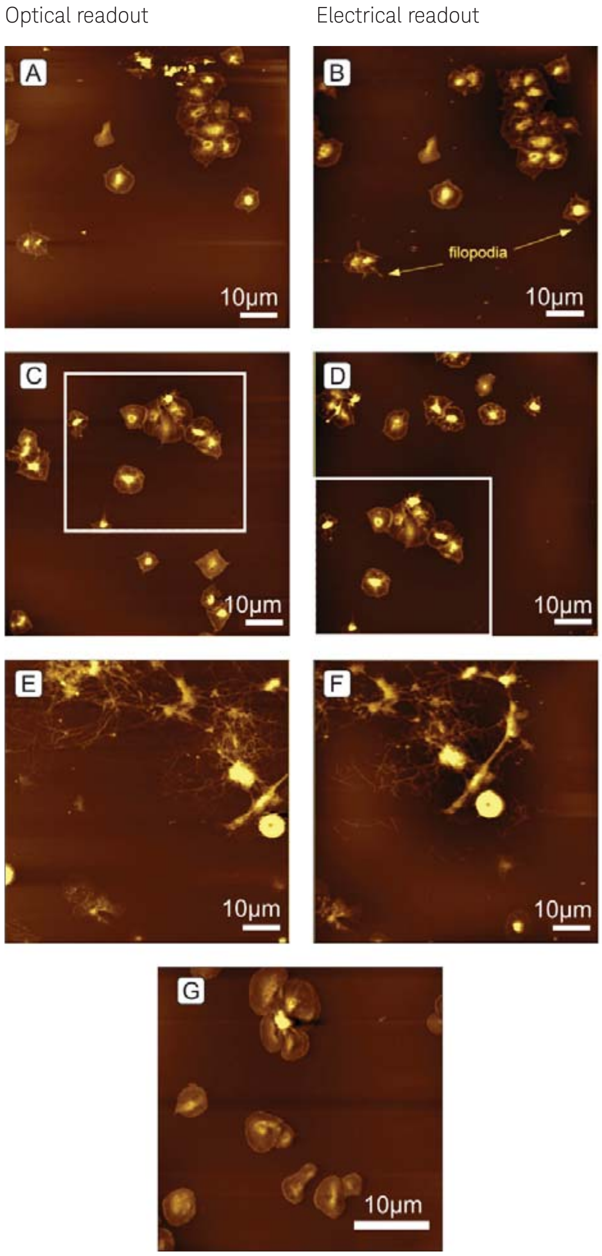

Figure 3. Comparison of electrical versus optical readout in a biological application. For (A-F) blood has been incubated to freshly cleaned glass slides for different time period (A and B: 1 minute, C and D: 3 minutes, E and F: 5 minutes). Afterwards the samples were carefully rinsed with PBS buffer and gently fixed and dried with nitrogen before imaging. For each sample an electrical and an optical readout image at the same sample location is given (as marked in C and D). (G) A one-minute sample image recorded in standard PBS buffer using self-sensing cantilever readout in intermittent contact mode (acoustic excitation). Z scale: 250 nm.

In terms of biology and medical relevance, different steps in the activation cascade of the platelets could be clearly resolved. After one minute of incubation (Figure 3A and B), the platelets show the expected disc like structure and the beginning of the formation of pseudopodia as shown previously [9]. The formation of pseudopodia is more prominent in the 3-minute sample, and a massive platelet aggregation, partly mimicking blood coagulation, can be seen after 5-minute incubation. This perfectly demonstrates the capability of the self-sensing cantilevers as a promising alternative solution that can simplify the preparation workflow.

Summary

The proof of principle images of the calibration grids and platelets in dry state and/or in liquid clearly demonstrate the capability of working with self-sensing cantilevers on a commercial AFM system, with just a minimum of additional hardware and without changes to the original setup. In general, this represents a huge step forward in applicability and advanced user experience, especially for non-frequent AFM users. As there is no need for laser adjustment or for thermal equilibration when working in liquid, therefore the preparation work flow is simplified and the time needed to get the first image of a sample is decreased significantly. In addition, less space limiting hardware is necessary as the whole optical detection system is obsolete. This increases the possibilities of combining AFM with other techniques of interest, including SEM, typically SEM-integrated equipment such as gas injection systems, nanoindenters [10], a tensile stage as currently performed at GETec Microscopy (http://www.getec-afm.com/index.php?id=15), as well as a combination of AFM with an inverted light microscope that raises the opportunity to combine self-sensing cantilever technology with fluorescence microscopy. Moreover, the images recorded in milk clearly indicate the possibility of working in non-transparent liquids, which might be of high interest especially for bio applications.

Acknowledgement

This work was done by Michael Leitner (michael.leitner@sclsensortech.com, Principal Investigator) and Alexander Deutschinger of SCL-Sensor.Tech. Fabrication GmbH, Boris Buchroithner and Andreas Ebner of Johannes Kepler University Linz in close collaboration with GETec Microscopy GmbH Franz Hofbauer.

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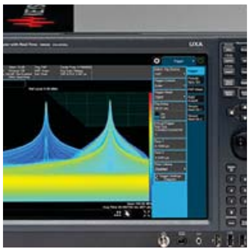

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