Correlative SPM, Raman and SEM analytic of biomedical devices and coatings

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INTRODUCTION

A variety of medical implants and microelectrode arrays for electrophysiology are fabricated in thin film and micro technology. To guarantee the quality, proper functionality and safe operation of these devices, analytical techniques to investigate the structure and chemical composition of surfaces and interfaces during the fabrication process and for final quality control are essential.

In atomic force microscopy a sharp tip is scanned over a sample surface and simultaneously a feedback signal is measured to control the tip-sample distance as well as to determine various surface properties. Due to different available feedback mechanisms, AFM not only allows imaging of surface topography and roughness, but reveals a variety of other surface properties including stiffness, friction and adhesion forces. Hence, AFM is suited to investigate surface topography and residuals after dry etch processes, to study functional layers on electrodes or to detect defects in insulation coatings. The missing channel in AFM is chemical information.

Raman spectroscopy provides spectroscopic information of chemical and physical properties of a variety of organic and inorganic materials as well as chemical composition of compound samples. The excitation of molecular vibration states by monochromatic light results in a specific energy change called Raman shift, which results in a spectrum acting as 'fingerprint'. Thus, Raman spectroscopy/microscopy allows investigation of interfaces in multilayer structures, probing homogeneity and

structural state of polymeric layers and identifying residuals or impurities after clean room processes with a lateral resolution of approximately 0.3 µm.

Tip-enhanced Raman spectroscopy represents an addition to conventional Raman measurements, utilizing plasmonic structures to induce nano 'hot spots' which locally enhance the electrical field. With optimized tip – sample settings, signal amplification of at least an order of magnitude can be observed, achieving nanometer resolution at the same time. TERS allows detection of single molecules or thin layers of residuals at high spatial resolution, which cannot be identified with any other technique.

Last, focused ion beam / scanning electron microscopy (FIB / SEM) in combination with energy dispersive x-ray spectroscopy is applied to image surfaces, cross sections and elemental distribution, suitable to verify layer compositions of encapsulated actuators and sensors in implants. All the SEM images in this work were acquired with a Carl Zeiss Auriga 40.

All mentioned techniques are correlated with each other to superimpose the acquired signal and performed analysis obtaining a maximum of relevant information. In this paper we present our Bruker – Renishaw AFM-Raman setup and two selected applications: a flexible microelectrode array (FlexMEA) with carbon coated electrodes used for local electrical stimulation of biological tissue and a novel active middle ear implant used for mechanical stimulation of the inner ear (cochlea).

FIGURE 1 Raman with encapsulated microscope and AFM setup

MATERIALS AND METHODS
SET UP

All measurements are performed using a Bruker Innova SPM in combination with a Renishaw inVia Raman spectrometer. The SPM offers the possibility of contact mode and non-contact tapping mode AFM as well as scanning tunneling microscopy (STM). Important features are the fixed probe – moving sample arrangement with up to 100 µm scan fields and an additional stage height sensor. The Raman spectrometer offers two excitation lasers (HeNe 633 nm and Nd-YAG 532 nm), streamline acquisition for fast spectra recording on large sample volumes and an extensive analysis software bundle.

Both systems are coupled by an optical bridge which is controllable in three dimensions with micrometer precision. The Raman laser is focused on the very end of the AFM probe with a

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FIGURE 2 SPM Head with 50x objective for focusing the Raman laser on the SPM probe
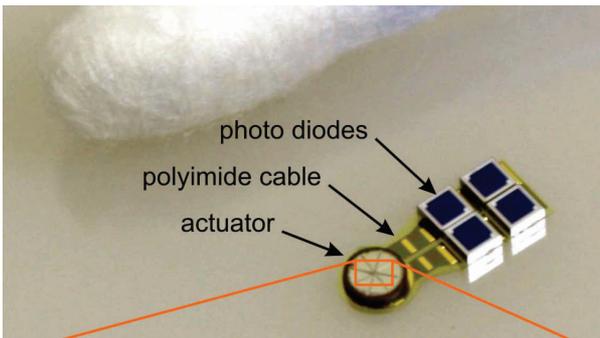
FIGURE 3 Schematic representation of the working principle of an active middle ear implant with optical transmission of the acoustic signal [1].
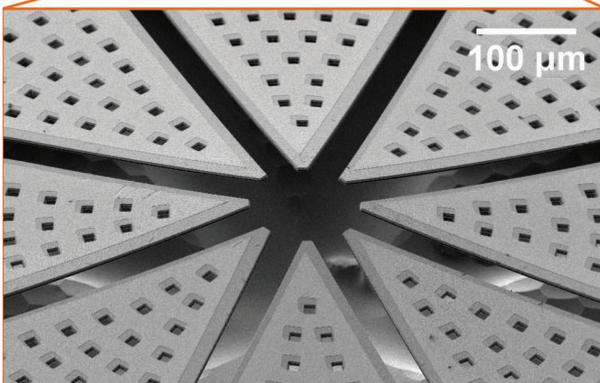

FIGURE 4 SEM image of the piezo actuator used for direct mechanical stimulation of the auditory system. The cotton bud in the upper picture is given for size comparison

FIGURE 5 Cross section of a Parylene-coated actuator membrane. The cross section was prepared using focused ion beam milling.

50x 0,45NA Objective in 60° reflection geometry. Thereby, it is possible to record Raman spectra while performing AFM scans and to achieve TERS signals using appropriate tips.

APPLICATIONS
APPLICATION 1: ACTIVE MIDDLE EAR IMPLANT

Various types of hearing aids are available for the treatment of hearing loss and deafness. The underlying working principle of most of these devices is the amplification of sound via a combination of a microphone, a speech processor and a loudspeaker. Yet, these conventional types of hearing aids are only applicable up to a certain degree of hearing loss. For more severe types of deafness the usage of active middle ear implants is indicated.

The new type of implant combines optical transmission of the acoustic signal to the inner ear with direct mechanical stimulation of the latter [1, 2]. The objectives of this approach are an easy implantation technique and a reliable coupling of the stimulation device to the inner ear.
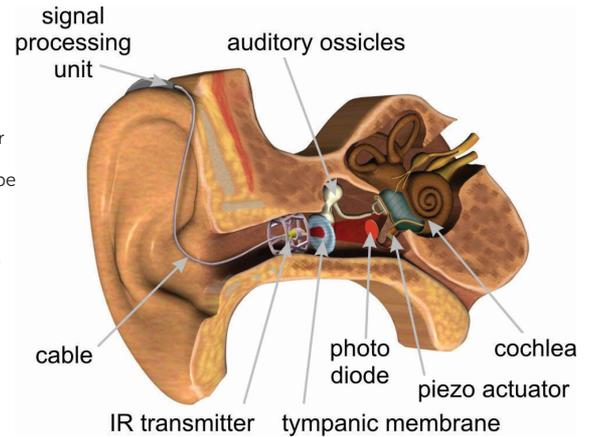
The working principle of this implant is shown in figure 3 schematically. A behind-the-ear unit records the sound, processes the signal and transfers it to an infrared diode that is placed in front of the tympanic membrane. A single photo diode or an array of photo diodes located in the middle ear transforms this optical signal into a voltage signal that is used to drive a piezo actuator, figure 4. To ensure good coupling of the actuator to the auditory system it is positioned directly in front of the round window membrane which seals the cochlea towards the middle ear. The electrical connection between the photo diode and the actuator is established via a thin and flexible flat cable comprising gold leads, gold contacts and a polyimide encapsulation. This polyimide cable is an in-house fabrication while the photo diode is commercially available and the actuator is application-specifically manufactured by of one of our project partners. The photo diode and the actuator are mounted on the flexible cable using conductive glue.

The actuator shown in figure 4 is fabricated on a silicon substrate and consists of a thin segmented flexible membrane that is covered with lead zirconate titanate (PZT) featuring piezoelectric behaviour. The top and bottom electrodes of the piezoelectric layer are fabricated from platinum and gold, respectively. When a voltage is applied between these two electrod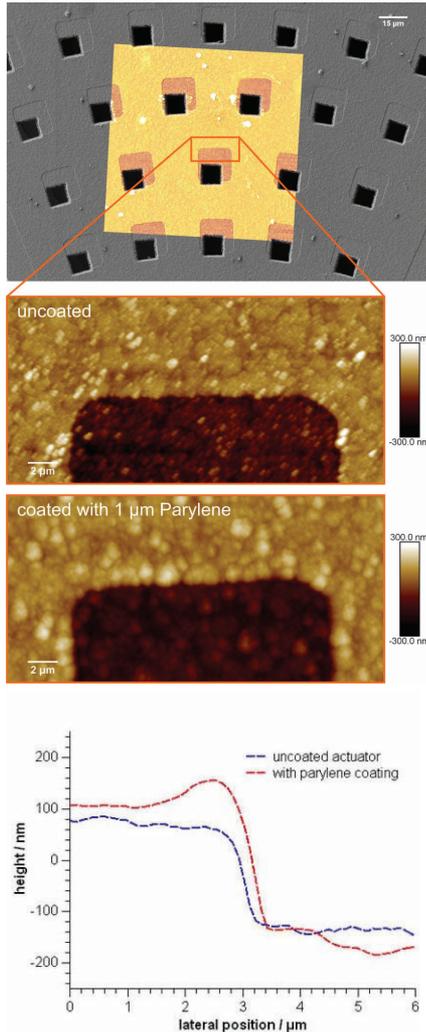es an electric field across the PZT layer is generated that leads to a bending of the membrane. This displacement is used for the mechanical stimulation.

To ensure proper function within the wet environment of the human body the whole implant needs to be encapsulated. For this purpose only flexible thin film encapsulations are suitable that do not reduce the vibration amplitude of the actuator significantly. Furthermore, the encapsulation material must show sufficient bio stability and biocompatibility ensuring that the implant can remain implanted in the human body for several years without loss of function. The encapsulation must not be dissolved or delaminated when it is exposed to fluids present in the body and foreign body reactions due to the implant must be avoided, respectively.

Parylene layers fabricated via chemical vapour deposition (CVD) in thicknesses of about 1 µm satisfy the above criteria. This process starts with thermal cracking of a precursor into monomers. These molecules are fed into a vacuum chamber where they adhere to all surfaces exposed to the gas and react to a polymer. The possibility to ensure conformal coatings on all kinds of sample geometry is the most notable benefit of this process [3, 4]. Beyond that the resulting layers are transparent which means that this coating can also be applied to the photo diode.

Figure 5 shows a cross section prepared from an actuator coated with Parylene. All surfaces of the membrane are covered with a homogeneous Parylene layer.

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FIGURE 6
Correlative SEM and AFM imaging of the membrane of a piezo actuator. AFM scans at higher magnification show a change in the surface morphology after the Parylene coating, as seen in the line profiles.

uncoated

coated with 1 µm Parylene

FIGURE 7
Raman spectra of a Parylene-encapsulated actuator. The red graph was acquired measuring above the gold electrode, the blue graph above the PZT layer.
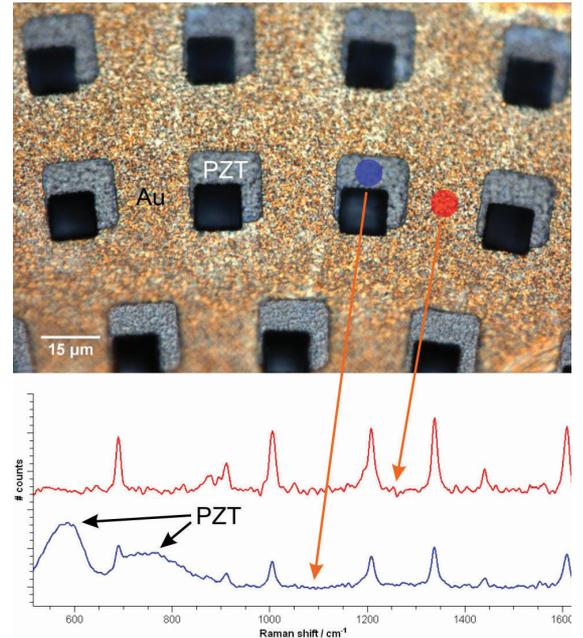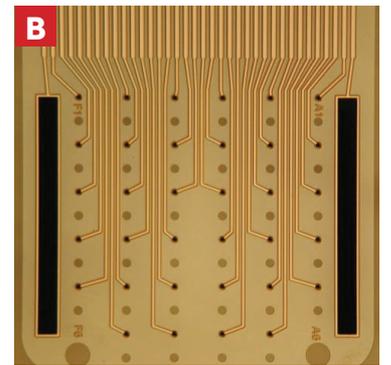

FIGURE 8 Flexible Micro Electrode Array: complete device (A) and overview of 6x6 array electrodes and two large reference electrodes (B)

AFM imaging, SEM imaging and Raman spectroscopy were used to control the encapsulation process. Figure 6 shows an overlay of an SEM and an AFM image of a segment on the piezo actuator membrane. The images were acquired sequentially and aligned afterwards using characteristic features that appear in both images as alignment marks.

AFM images with reduced field of view were acquired before and after the Parylene coating was deposited. The surface roughness visible on the uncoated sample is mainly caused by different grains of the polycrystalline PZT and gold layers. The Parylene coating smoothens this small-scale surface morphology while the main features of the sample like height steps at layer edges and openings in the membrane are preserved.

In addition to the AFM scans Raman spectra were acquired before and after the encapsulation process. The results are shown in figure 7. The sharp Raman peaks in the spectra are characteristic for Parylene but due to its low thickness peaks originating from the underlying material can also be registered. The spectra of the Parylene on top of the PZT layer shows additional peaks for wave numbers between 500 and 800 cm-1 which can be related to the PZT ceramic. No additional Raman shifts were observed above the gold layer in the depicted 500 to 1600 cm^{-1} range.

APPLICATION 2: FLEXIBLE MICRO ELECTRODE ARRAY (MEA)
Flexible MEAs [5] are used for in-vitro and in-vivo electrical stimulation or recording of extracellular signals of neuronal cells, in brain tissue and also of cardiomyocytes in heart tissue. This device consists of thin conductor lines buried in a flexible insulator such as polyimide, and electrodes with a diameter in the range of 30µm to 500µm which are arranged in an array, for example a 6x6 configuration with spacing of some 100µm. Titanium nitride is used as electrode material. In a new approach carbon nano tube (CNT) like electrodes are fabricated. A low temperature chemical vapor deposition (CVD) process enables growth of CNTs electrodes embedded in polyimide substrates [6]. Figure 8 shows one example and a typical layout of the micro electrodes on a flexible substrate. In Figure 9 light microscope, Raman and SEM analysis of a single electrode are summarized.

AFM is used to control the surface roughness, step height and potential defects of the micro electrodes and the surrounding insulating polymer.

Spectroscopic imaging of the same electrode in the inVia Raman microscope is shown in figure 9. The Raman spectrum of the deposited carbon layer has peaks at 1380 and 1600 cm^{-1} which can be related to D and G bands of carbon. The broad peaks in the spectra are an indicator that this carbon layer is a mixture of pure CNTs and different phases of carbon.

For future development of transparent electrodes we use transparent ITO (indium tin oxide) tracks to connect the micro electrodes. The electrodes are made of thin carbon layers. These thin carbon layers can only be detected by TERS as the TERS effect provides very localized enhanced signal. In general, carbon-based materials like graphite, carbon nanotubes and
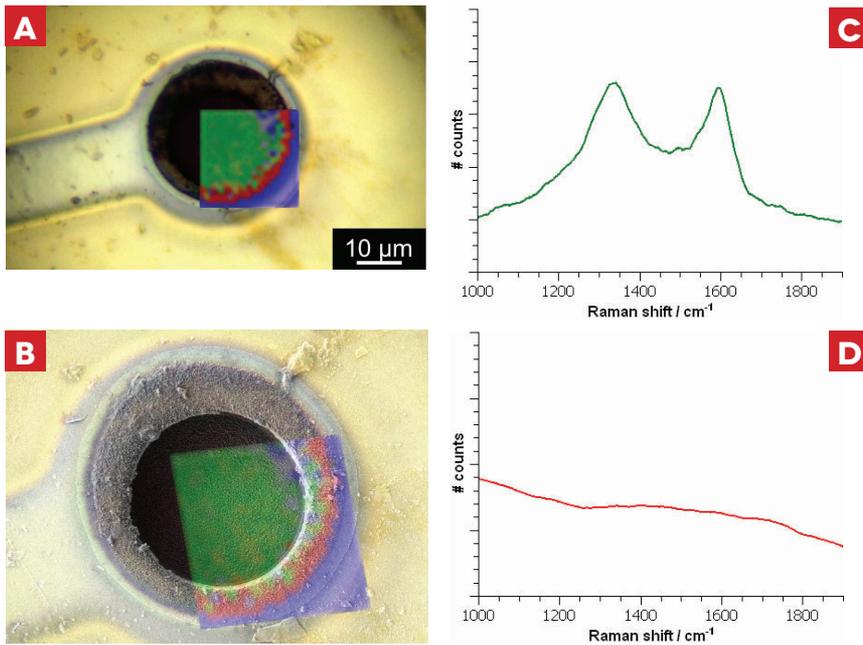
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FIGURE 9 Overlay of light microscope (A), SEM image (B) and Raman mapping. Broad D and G peak (C) show a mixture of amorphous carbon and carbon nanotubes. High fluorescence at the border of the micro electrode (D). Increased signal (blue) in the surrounding polyimide insulator.
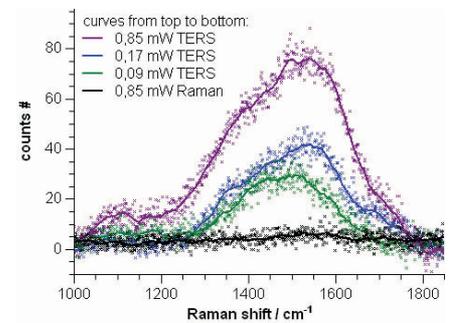

FIGURE 10 TERS measurements on a 10 nm thick carbon layer with different laser intensities in comparison to conventional Raman spectroscopy (black line). 20 accumulations./1sec, STM: 1nA, 1V bias
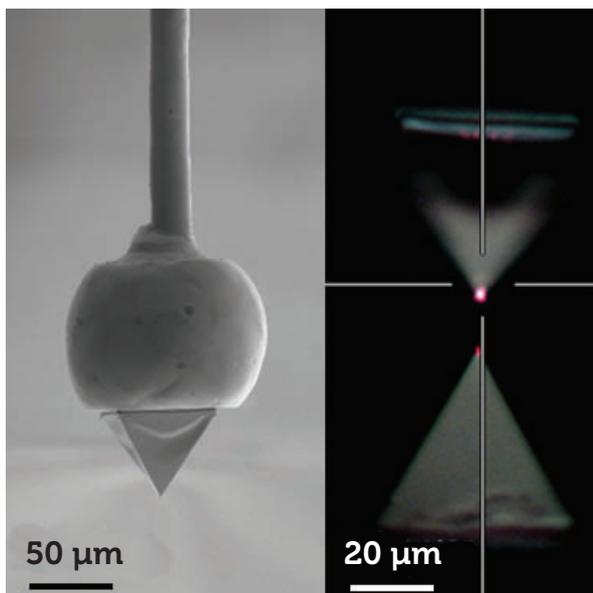

FIGURE 11, STM TERS Probe: Gold pyramid bonded to a wire (left). Raman laser focus on the pyramid tip (right)
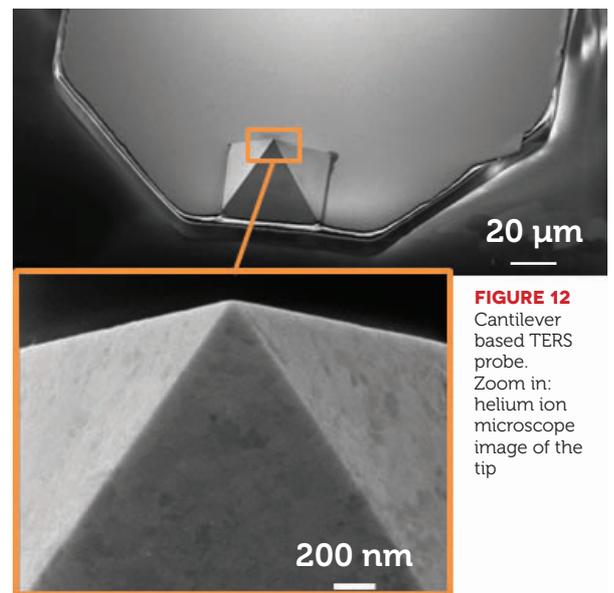

FIGURE 12 Cantilever based TERS probe. Zoom in: helium ion microscope image of the tip

graphene present promising candidates as electrode material. For the benefit of transparent layers, a homogeneous coating with a thickness in the nanometer range is required.

In a first approach we fabricated thin carbon layers on an electrical conducting metal layer by PVD deposition. The thickness of the generated layer is controllable with a QCM (quartz micro balance) and ultra-thin layers can be prepared reliably. Figure 11 shows TERS measurements on a 10 nm thick carbon layer using the newly-developed STM-TERS probes described in the next section. With conventional Raman it is challenging to obtain a signal from the carbon layer, however when using TERS the spectrum can be easily seen. This demonstrates the significant

surface signal enhancement provided by TERS and its benefit to measurement sensitivity. TERS also allows spectra to be collected using low laser energies, thus preventing radiation damage of the surface. Measurement parameters were 20 accumulations/1 sec, STM: 1nA, 1V Bias.

DEVELOPMENT OF NEW TIPS FOR TERS

In recent years, tip-enhanced Raman spectroscopy has pushed more and more from fundamental research into routine application. Besides instruments which fulfill the physical requirements for TERS, the supply of adequate probes is still limited. Therefore, a part of our work is focused on the development and characterization of STM-TERS needle

probes and AFM cantilever probes.

Recently, a template stripping process has been suggested to batch fabricate highly reproducible pyramid-shaped tips with outstanding plasmonic properties [7]. Following this approach and optimizing the fabrication process we are able to achieve a high yield of probes with similar performance. These tips, seen in figure 11, can be attached to different support structures and thus are usable in almost every STM-TERS imaging system.

Additionally, cantilever-based probes are necessary to expand AFM-TERS application to all materials: conductive and also insulating samples, like ceramics. A fabrication process for cantilevers featuring the same tip properties as discussed before is
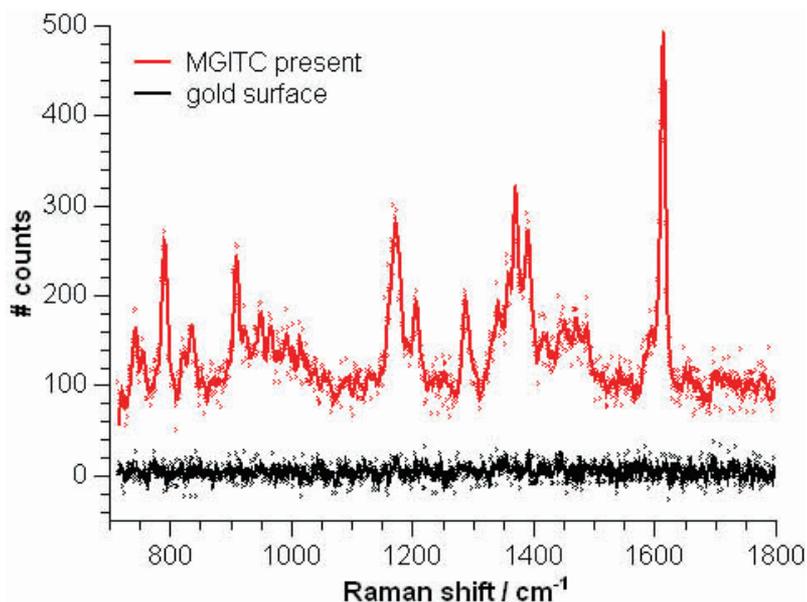
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FIGURE 13 TERS spectra of MGITC

currently in development and first prototypes are available, figure 12.

TERS CONTROL MEASUREMENTS

A standard functional test in the field of tip-enhanced Raman spectroscopy is the measurement of a photo active molecule, e.g. Malachite green isothiocyanate. Samples are commercially available from Bruker (TERS-SMPL) or can be fabricated by immersing a surface in a diluted solution of dye and rinsing it with ethanol.

Figure 13 shows recorded tip-enhanced Raman spectra of a MGITC functionalized gold surface. The developed pyramidal gold tip is scanned over the sample using STM operation mode. With precise laser alignment onto the very tip of the probe, characteristic MGITC spectra with high intensity can be measured reproducibly with resolutions at tens of nanometers, corresponding to a few molecules.

SUMMARY AND CONCLUSIONS

Correlative AFM, Raman and SEM analysis provide information about structure and chemical composition of material surfaces and interfaces with nanoscale resolution. With tip enhanced Raman spectroscopy (TERS) detection limits of Raman spectroscopy can be improved. Very thin amorphous carbon layers prepared by physical vapor deposition (PVD) can be much more effectively detected in TERS. These analytical tools were successfully used to analyze ceramics, polymer and carbon layers on biomedical devices. For TERS we have developed reproducible pyramidal gold probes. In future passivated silver probes will also be available.

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ABSTRACT

Raman spectroscopy can add chemical information to scanning probe microscopy (Atomic Force Microscopy, AFM or Scanning Tunneling Microscopy, STM). Optical coupling of a Raman spectrometer to the tip of a SPM instrument enables the recording of Raman signals from surface areas near the probe tip. With this combined instrument three different operating modes are possible: (1) correlative SPM and Raman, (2) co-localized SPM and Raman and (3) TERS (Tip-Enhanced Raman Spectroscopy). To complete this correlative nano analytic approach, survey imaging with SEM (Scanning Electron Microscopy) was performed. These nano analytical tools were used to investigate the surface structure and chemical composition of components for biomedical devices (active middle ear implant, flexible electrode arrays) and coatings (polymers and carbon). For TERS operation novel probes have been developed showing highly sensitive, reproducible detection of ultrathin carbon layers in STM-TERS mode.

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