II.1.2 Nanoscale Electrochemical Probes for Monitoring Bioconversion Hydrogen

Investigators
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Introduction

The economic production of hydrogen remains one of the key issues for realizing the hydrogen economy. In response to this challenge, Professors Swartz and Spormann, are pursuing biological production of hydrogen as part of the GCEP hydrogen initiative. Our team works with Professors Swartz and Spormann to develop nano scale sensors to better understand the electro-chemistry of hydrogen production via bio-energy conversion. The sensors are designed to measure reduction-oxidation reactions, electron transfer reactions, and the broader kinetics of biochemical processing within the cell cytosol. The sensors we are pursuing may also be employed to determine membrane potentials across lipid bi-layers. Considering the size of the cells we are investigating, which is less than 5 microns, we estimate that the size of the sensors must be at least one order of magnitude smaller. Therefore, we manufactured probes with a diameter of tens to hundreds of nanometers. We are integrating these sensors into a single cell diagnostic platform that is capable of mapping biochemical reactions inside single cells. (A schematic drawing of the experimental set up is shown in Fig.1).

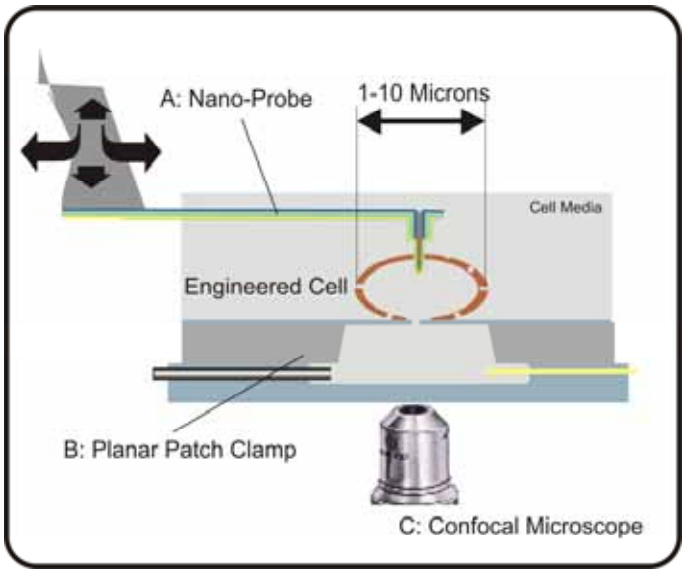

Figure 1: Single cell diagnostic platform with electrochemical nanoprobes,
A: Electrochemical nanoprobes are mounted to an atomic force microscopic stage,
B: Localization of single cells is achieved with a planar patch clamp device,
C: For optical visualization a confocal fluorescence microscope is used.

Background

The impetus for the development of dynamic intracellular sensing probes comes from an increasing body of evidence substantiating the role of intracellular ion flux in cell metabolic control. Observing the dynamics of intracellular electrochemistry is crucial in creating a theoretical framework for understanding interactions between signaling mechanisms and reaction paths of bio-energy conversion within a single cell and also between networks of cells.

Today, the highest resolution tools investigating cell communication phenomena are cell attached capacitance measurements using patch clamps. The revolutionary insights provided by the 'patch clamp' sensor of Sakman, Neher, and Marty [1,2] furthered our understanding of transmembrane signaling mechanisms. However, still there is no capability to investigate electrochemical phenomena simultaneously at a variety of different cell locations and couple that data with force feedback measurements.

The purpose of these efforts is to develop a probe capable of electrochemically characterizing the cell membrane surface and the cell interior by piercing the cell while causing minimal damage. Previous research identified Scanning Electrochemical Microscopy (SECM), as an important analytical tool for studying surface reactions and their kinetics down to nano-scale dimensions. The use of this method has been demonstrated in a wide range of applications, such as resolving fast heterogeneous kinetics at various material interfaces or imaging of biological molecules. [3-5] The spatial resolution of SECM depends on shape and size of the electrochemical electrode. Ultra Micro Electrodes (UME), which are tip probes having sub-micron electrodes on the top, are required to obtain resolution at the nanometer scale. Manufacturing approaches have been investigated ranging from isolation of etched metal wires or Scanning Tunneling Electron Microscopy (STEM) tips for single electrode systems to batch fabrication strategies for electrode array systems.[6-9] A combination of SECM with other Scanning Probe Microscopy (SPM) techniques, such as Atomic Force Microscopy (AFM), is highly desirable in order to obtain electrochemical information as well as complementary surface information simultaneously. [10] In particular, the combined use of SECM and AFM will allow for precise positioning of probes adjacent to or inside of cells and sensing of concentrations and fluxes of electrochemically active substances. A crucial component of such a system is the specialized probe system that has to be composed of a micro-mechanical cantilever structure necessary for the AFM mode and an electrochemical UME-tip required for a high performance SECM. Several strategies for fabrication of such transducer structures have already been reported. [11-16] While these efforts resulted in functional tip structures and combined AFM and SECM imaging, performance issues related to the tip sharpness, low aspect ratio of the tip structure, and size of the electrochemical electrode remain. The commonality for most of the above described fabrication technologies is a single electrode production scheme, which limits miniaturization potential and fabrication of multi-electrode systems. In order to overcome these limitations, enable radical miniaturization, and build high density array probes, fabrication schemes exploiting micro- and nano-fabrication technologies have been developed.

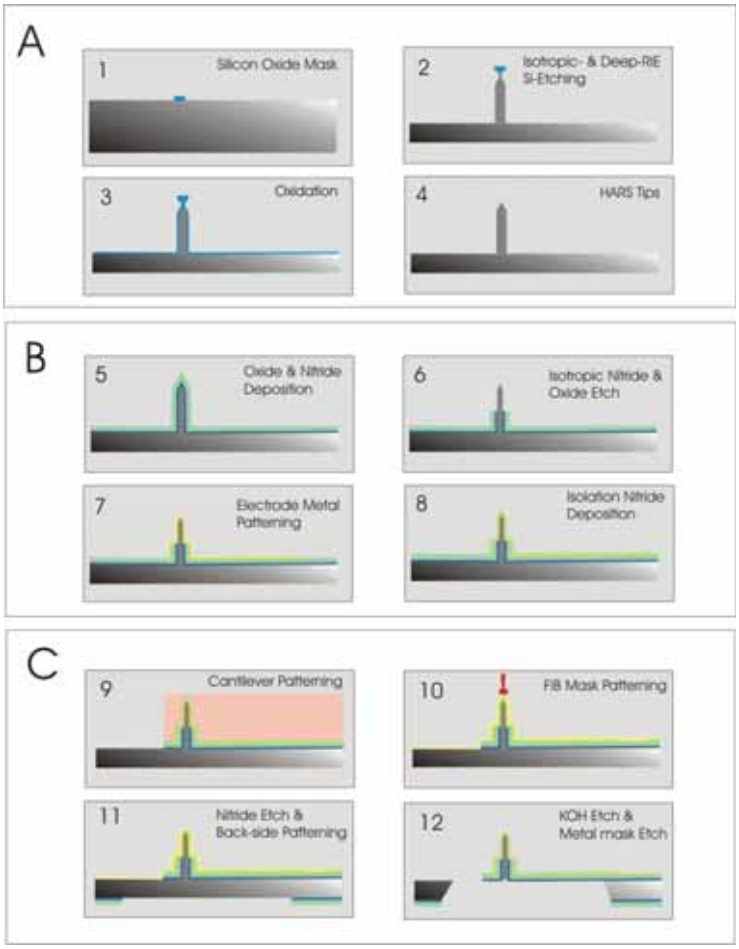

Figure 2: A) Fabrication of HARS tips: 1 Patterning of a silicon oxide etch mask, 2 Isotropic silicon etching to shape the tip and Deep-RIE Si-Etching to shape the shaft, 3 Releasing silicon oxide caps and sharpening of tips by oxidation and back etching of silicon oxide, 4 finished HARS tip, B) Embedding of HARS tips in silicon nitride and metal patterning of the electrode system: 5 Growing of silicon oxide and silicon nitride, 6 Back-etching of the silicon nitride using non uniform resist coating on high- aspect ratio structures and release of oxide layer using wet etching, 7 Patterning of the electrode system utilizing lift-off technique, 8 Isolation of the electrode system and tip structure with deposition silicon nitride. C) Fabrication of UME's and cantilevers: 9 Shaping of cantilevers, 10 FIB-patterning of etch metal mask, 13 Etching of the isolation layer exposing the electrode metal layer (Creation of UMEs) and patterning of back side etch mask for release, 12 Releasing of cantilever structure using wet etching.

Results

Micro- and nano-fabrication technologies originate from the field of microelectronics, combining parallel processing techniques with a miniaturization potential of sub micron regimes. In particular, high-aspect ratio electrochemical tip probes embedded in silicon nitride cantilevers have been developed for simultaneous AFM and SECM analyses.

[9,17] The fabrication process is based on batch processes in combination with an etch-mask technology utilizing FIB techniques to achieve both well defined UME and sharp high-aspect ratio tips on a single cantilever as well as in cantilever arrays. The process has been developed on four-inch wafers and is divided into three main fabrication pathways. An overview of the fabrication process is depicted in Fig. 2.

First, High-Aspect Ratio Silicon (HARS) tips are shaped combining isotropic etching with an anisotropic Deep-RIE-silicon etch process. Second, silicon tips are embedded in a silicon nitride layer, electrode systems are patterned and passivated with an isolation layer. Finally, a UME on top of the tip structures is established by etching the isolation layer only at the tip. To achieve accuracy and resolution in the nanometer regime on the HARS tip, an etch mask technology has been developed utilizing a focused ion beam-based technique. Subsequently, the cantilevers with embedded electrochemical tip electrodes are shaped and released. A detailed description of the whole fabrication process can be found in previous publications.[9,17]

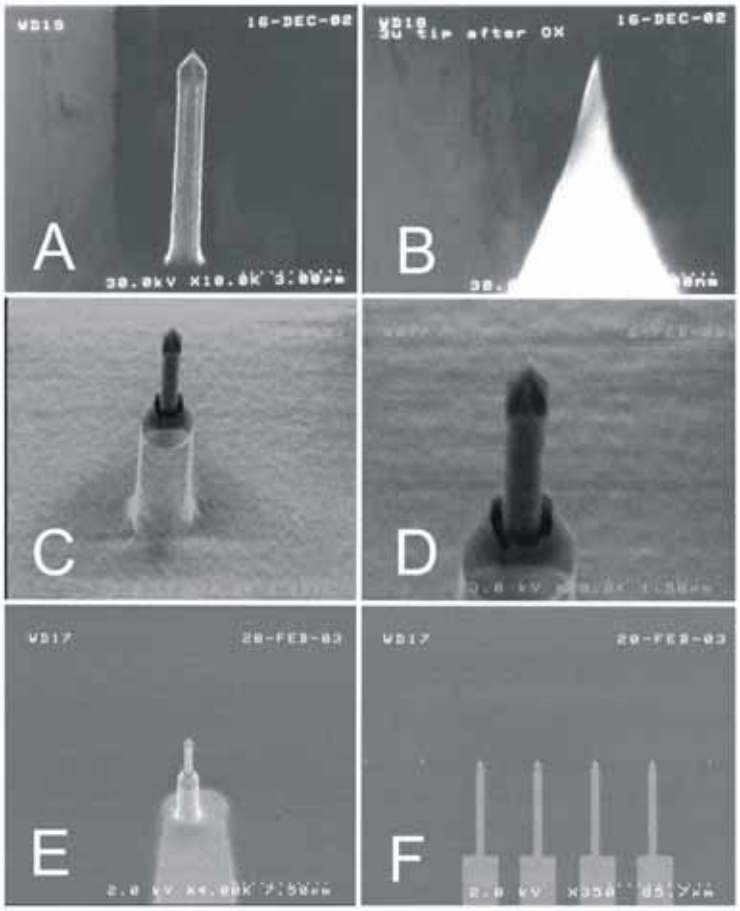

Figure 3: Tip-probes at different fabrication levels A) Example of an etched HARS tip B) Detail of a sharpened tip with a tip radius smaller than 50 nm C – D) HARS tips embedded in a 500 nm thick low stress silicon nitride E - F) Metallized embedded HARS tips.

In Fig. 3A, an example of a finished HARS tip is shown. Tips with a shaft diameter of 1.2 µm are created using oxide caps as a mask.[9,18] HARS tips with an aspect ratio of up to 20 and a diameter in the sub micron regime can be fabricated in this way. Fig. 3B shows a detail of a sharpened tip with a tip radius smaller than 50 nm. HARS tips embedded in a 500 nm thick low stress silicon nitride are shown in Fig. 3C and D. Application of a non-uniform coating of photo resist in combination with silicon nitride etching allows for the exposure of the silicon tips. [17,19] About 5 µm of the silicon tip at its base is anchored in the silicon nitride while the rest of the tip protrudes from the nitride layer. This processing step results in an additional reduction of the tip diameter to 600 nm and creates further sharpening of the HARS-tip.[28] In Fig. 3E and F, a platinum electrode structure is patterned on the embedded tips. Using magnetron sputtering, a homogeneous metal layer is formed and sufficient side-wall coverage of the HARS tip structures is observed.

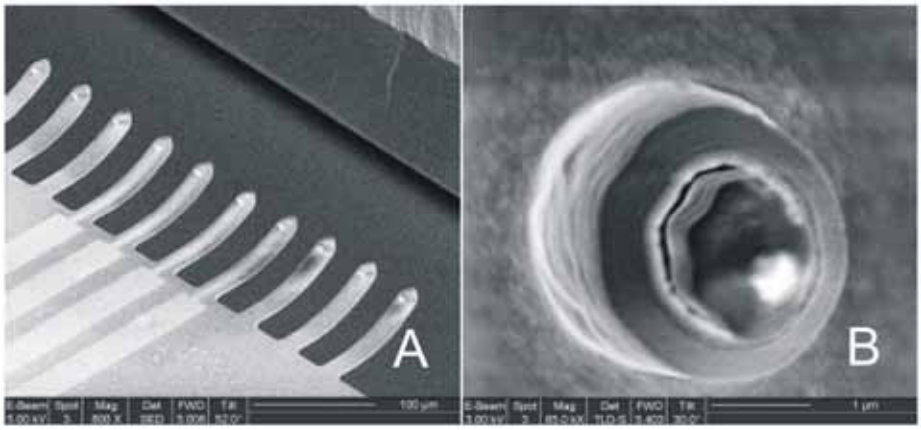

Figure 4: SEM pictures of a finished tip probe array A) Tip probe array with four UME cantilever structures B) Tip probe of the cantilever structures with a platinum UME (radius of 200 nm).

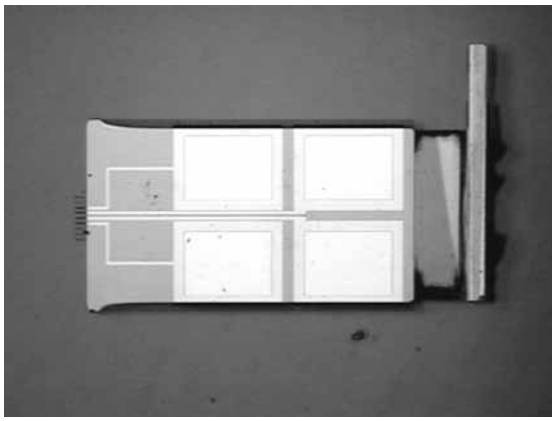

Figure 5: Optical low magnification micrographs of an SECM-AFM probe device.

This conformal metallization establishes a reliable electrical connection between an electrode on top of the tip and bonding structures on the bulk silicon. This process is not limited to single electrode patterning and can be expanded to larger linear arrays as well as to two-dimensional arrays. UME's on top of the tips are recognizable in Fig. 4B. The UME diameter of these probes is about 200 nm. A finished tip probe array is depicted in Fig. 4A. The bending of the cantilevers is caused by small residual stresses resulting from the nitride thin film passivation layer. The probes are used for the electrochemical characterization. A low magnification optical micrograph shows a probe device with an array of four cantilever tip probes and its bonding pad system (Fig. 5).

Electrochemical Characterization

Electrochemical characterization is carried out using a Solatron 1287, an electrochemical interface, in combination with a Solatron 1260, Impedance/Gain Phase Analyzer (Solatron Analytical). As shown in Fig. 6, a three-electrode arrangement was employed. The cantilever device is mounted on a micromanipulator stage (PCS-6000, Burleigh Instruments) or Atomic Force Microscope (Picoplus, MI). The tips on the cantilever are immersed and positioned in a drop or film of electrolyte in a controlled fashion. A platinum thin film layer is used as a counter electrode and an Ag/AgCl wire electrode functions as the reference electrode. Cyclic voltammetry of the tip probe electrode with a platinum wire reference electrode is used to characterize the electrochemical behavior of the tip probes. In these experiments, phosphate buffer is used as an electrolyte solution.

The tip probe system shows all the important electrochemical surface reactions of a platinum electrode in a phosphate buffer solution. Hexaammineruthenium (III) chloride is used to study the response of the electrode in a reversible redox system. Fig. 7 shows voltammograms taken in an electrolyte with 10mmol and 0mmol concentration of $Ru(NH_3)_6^{3+}$. The tip probes in these experiments have an approximate electrode area of 0.125 μm2. The reduction current appears in the potential regime below –200 mV related to Ag/AgCl at 0.1 mol KCl. Electrochemical Impedance Spectroscopy (EIS) analysis leads to a faradic impedance of the tip transducer system at a DC working potential of -300 mV of 5×10^7 Ohm for 10mM $Ru(NH_3)_6^{3+}$ and 4×10^9 Ohm for phosphate buffer only. Both, the behavior of the reduction current and values of the faradic impedance in a $Ru(NH_3)_6^{3+}$ redox system shows typical characteristics of a single ultra micro electrode in sub micron size and indicate the electrochemical functionality of the tip probe system.

This electrochemical, platinum, nano-sized transducer system can be directly applied to measure ion fluxes or electrochemically active species within and between cells. Furthermore the transducer system can be modified to detect specific ions such as sodium, potassium, or substances involved in cellular metabolic reactions or signaling pathways.[21]

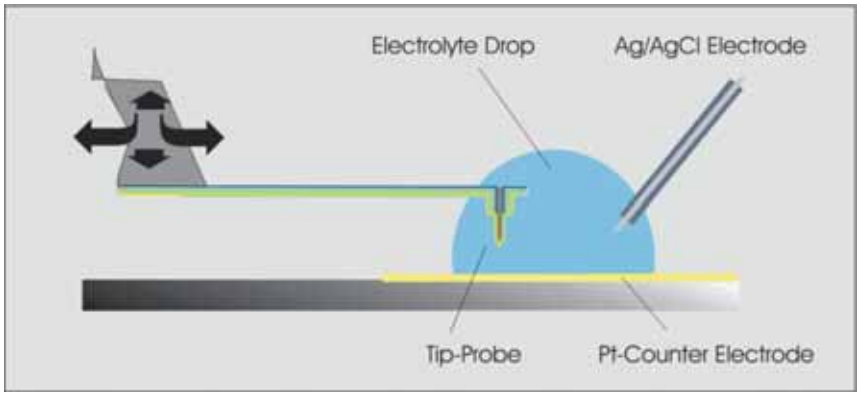

Figure 6: Schematic electrode arrangement of an electrochemical measurement set up.

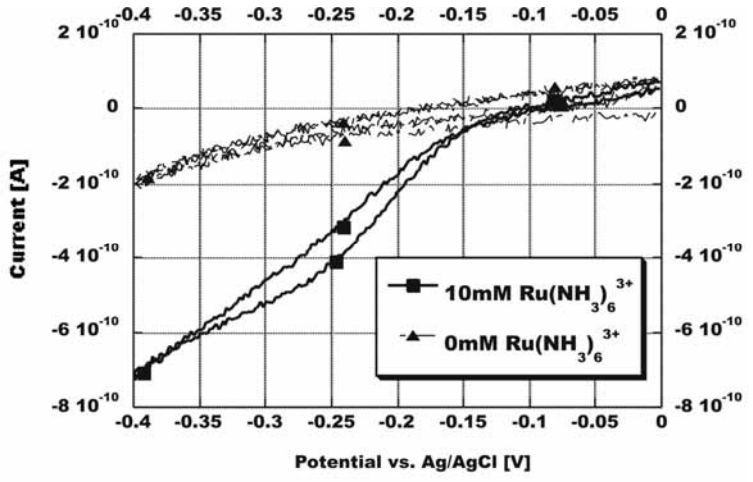

Figure 7: Cyclic voltammograms of tip probe with a platinum UEM (0.125 μm^2) in 0.1 M Phosphate buffer electrolyte and in a 10 mmol $Ru(NH_3)_6Cl$ and 0.1 KCl solution. The potential is related to a 0.1 M KCl Ag/AgCl-Reference electrode, Potential sweep rate was 10 mV/s.

Silicon nitride cantilever array with single tips and tip arrays

Obtaining measurements with high spatial resolutions requires increased probe densities. Arrays of multi-probe systems on a single cantilever were designed and fabricated as shown in Fig. 8. Silicon tips with spacings of 10 micrometers are embedded in silicon nitride pillars and supported by a 500 nanometer thick silicon nitride cantilever. The electrically conductive tips are connected separately to the monitoring system such that individual signals from each tip can be transmitted. The metal lines are sputtered on an isolated substrate surface and covered with another insulation layer to define the UME on the tip of the probe structure as described previously. The cantilever and its substrate are compatible with conventional AFM (Atomic Force Microscope) systems. The probe or probe array can be mounted onto an AFM scanner to take advantage of the precise closed-loop motion control and force feedback data of the AFM system.

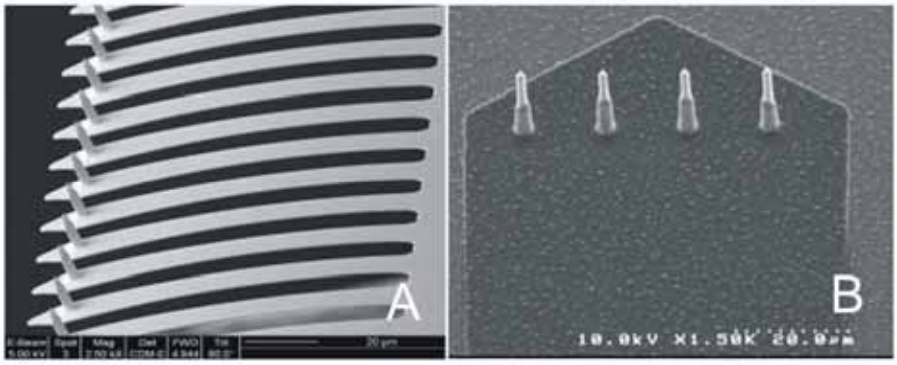

Figure 8: A) Silicon nitride cantilever array with single high-aspect-ratio tips. The thickness of cantilevers is about 500 nm B) High-aspect-ratio tip array on single silicon nitride cantilever.

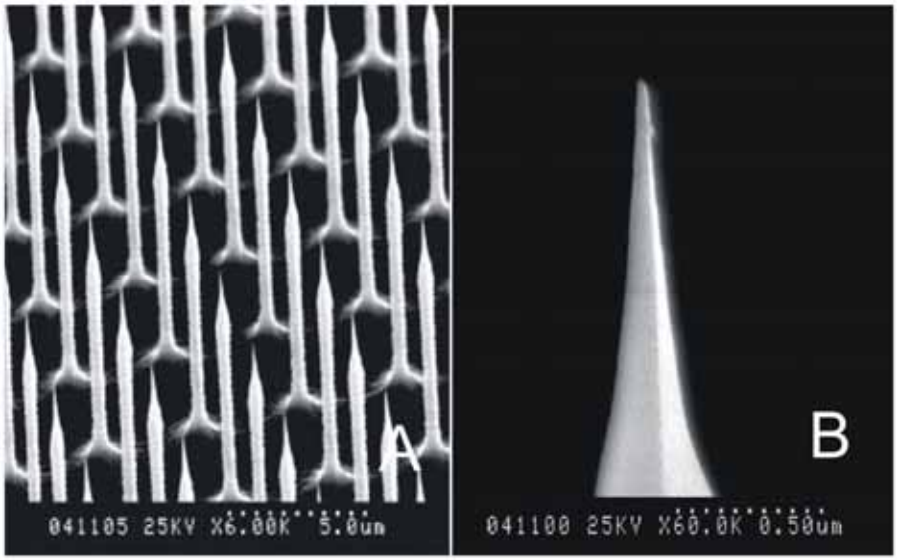

Figure 9: A) multi-array ultra sharp silicon tips fabricated with a combination of two plasma etching steps. B) a closer look of the individual silicon tip with a radius less than 20 nm

Multiple arrays of ultra sharp high-aspect-ratio silicon tips

Multiple arrays of ultra sharp nano-tips with tip radii as small as 20 nanometers are currently being fabricated by combining two plasma etching processes on single crystalline wafers. As shown in Fig. 9, nano-tips located on top of the pillars respectively have an aspect ratio of over 10:1. Such a high density of ultra sharp tips represents a potentially powerful tool for measuring cell properties in real time. The tips can be inserted into cells such that multiple electrodes are present within a single cell or with a

single tip in adjacent cells. The electrically active tips will be individually connected by conductive vias to CMOS transistors on the back-side of the wafer. In this way, both individual addressability and signal processing are achieved.

The tip is defined, so that most of the tip is insulated and only a small region at the top has an exposed metal electrode. This will provide a well defined tip geometry with nanometer dimensions to further assure localized measurements of dielectric behavior within the cell interior. The fabricated nano-tip arrays on cantilever structures integrate well with the AFM stage for precise positioning relative to cell surface and subsequent penetration of the cell membrane.

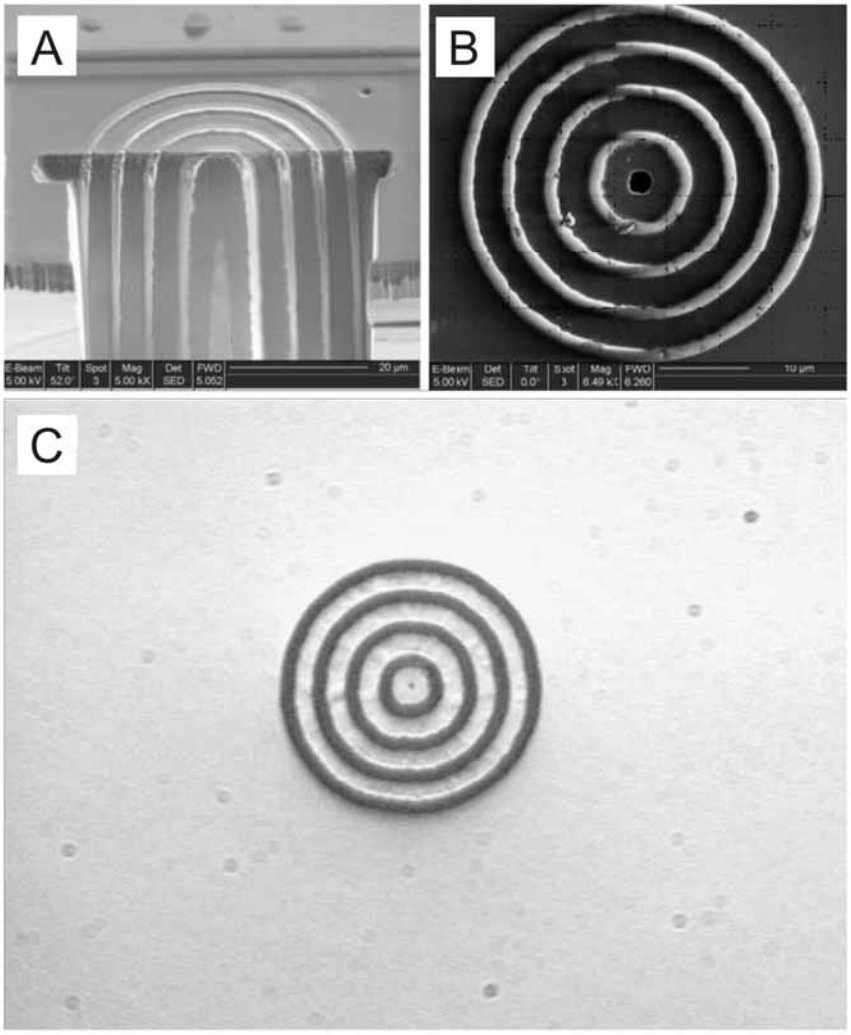

Figure10: A 40µm-diameter ETWI consisting of four 2µm-thick poly-silicon layers and four 1µm-thick thermal silicon-oxide layers A) SEM image of the cross section B) SEM image of the top view (poly-silicon and silicon appears as dark, silicon-oxide appears as bright) and C) Optical microscope image of the top view (poly-silicon and silicon appears as bright, silicon-oxide appears as dark).

Fabrication of silicon Via Structures

Limitations of connection density of current technologies are imposed by space restrictions resulting from designing and fabricating both connection structures (bonding pads, flip chip pumps) and functional devices (MEMS or CMOS) on a single wafer side. In order to overcome these limitations and open the use of both sides of a wafer independently, electrical through-wafer interconnections are necessary. The through wafer interconnection concept is based on multi-layer deposition techniques. Openings in a double-sided polished wafer are created by applying a high-density inductively coupled plasma (ICP) etch technique.[22] Hole structures with a diameter of 20 μm are formed through a 350-μm thick wafer. A multi-layer system of up to eight layers consisting of alternating conducting layers (N-type doped poly-silicon) and isolating layers (silicon-oxide) are grown until the vias are filled. The silicon-oxide and poly-silicon layers are grown using a low pressure chemical vapor deposition process to achieve a high deposition uniformity. Subsequently, all layers on the wafer surface are removed in a chemical mechanical polishing process. In this way, a multi-connection, through-wafer structure can be fabricated. Fig. 10 shows an example of such a multi-layered interconnection structure. The applied low-pressure chemical vapor deposition techniques guarantee a sufficient homogenous coating outside and inside of the entire structure to a minimum layer thickness of one micrometer. The connection quality has been examined combining impedance spectroscopy and focused ion beam technology. Depending on the geometry and the doping profile of the poly-silicon layers, a connection resistance of less than 80 Ohms can be achieved with sufficient DC isolation. This technique is compatible with high temperature processes and is suitable for MEMS as well as CMOS applications.

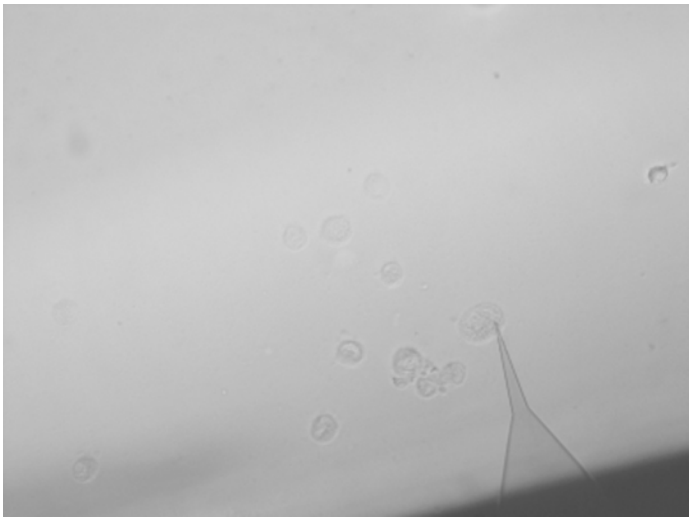

Figure 11: Ultra-sharp planer silicon nitride tip fabricated with a combination of micro-fabrication technique and FIB (Focused Ion Beam) techniques penetrating individual cells.

Progress

The developed probe systems can contribute to a fundamental understanding of cell signaling mechanisms and reaction schemes of bio-energy conversion at unprecedented levels of spatial and temporal resolutions. Fig. 11 shows an example of a tip probing individual cells with an ultra-sharp planer silicon nitride tip probe.

These unique analytical tools are applicable to a variety of important scientific questions related to energy conversion strategies without the production of carbon compounds as a by-product. One example is the investigation and verification of electrochemical reactions, electron transfer mechanisms, and their kinetics of genetically modified bacterium cells on a single cell level. The developed measurement set-up allows for an investigation of effects due to genetical modification of cells and helps to effectively engineer a cell system specializing in a clean generation of molecular hydrogen.

Further, the transducer system can be applied to study bio-energy conversion processes with a highest spatial resolution in cells or, ultimately, in energy converting cell organelles such as the mitochondria.

Furthermore, this developed probe system is not only applicable to the investigation of hydrogen generation processes but also applicable to the exploration of new technologies for hydrogen utilization. In particular, this probe system is well suited for studies of electrochemical conversion reactions and their opportunity in terms of efficiency and low cost of fuel cells at sub micrometer scale. These investigations are currently pursued for the GCEP project "Micro and Nano Scale Electrochemistry applied to Fuel Cells".

The development of this new class of electrochemical nano-probes enables us to provide a unique tool to explore reaction paths, characterize material properties at nano scale for both generation and utilization of hydrogen in a clean and efficient fashion.

Future Plans

An electrochemical cantilever transducer system with platinum electrodes in sub micron regimes was developed. Electrochemical investigations showed full functionality of the probe system. Due to the high aspect ratio topography of the tip structure and low spring constant of silicon nitride cantilevers, these probes are particularly well suited for combined high-resolution SECM and AFM analysis in living cells.

Next year the probe system in combination with the single cell diagnostic platform (Fig.1) will be applied for:

- Study of genetically modified cells for clean hydrogen generation.
- Study and exploration of energy conversion reaction in energy converting cell organelles such as mitochondria.

Further, the probe system will be applied for:

- Study of electrochemical phenomena at nanometer scale of fuel cell for an efficient and low cost hydrogen utilization.

Publications

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