# Intracellular Neuronal Recording with High Aspect Ratio MEMS Probes

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## ABSTRACT

Micro-machined silicon needles capable of penetrating through cell membranes were fabricated and tested for intracellular sensing applications. The fabricated needles have sharp tips (diameter <1 μm), they are long (>300 μm) and exhibit high mechanical strength. The needles were tested for extra- and intra-cellular neuronal recording applications. To prepare the needles for neuronal recording, they were coated with metal and their shanks insulated. Using these needles, we were able to obtain extremely localized extracellular signals and to perform first recordings with silicon based micro-probes from the *inside* of neurons.

**Keywords:** Neuronal-electrodes, Intracellular, Needles.

## INTRODUCTION

Extensive work in recent years has shown the potential of Micro-Electro-Mechanical Systems (MEMS) for neurological and electrochemical sensing applications. MEMS devices are particularly promising due to their small dimensions and the ease with which multi-site devices can be produced. Previous efforts in MEMS probes for neurological and electrochemical sensing for biological applications focused on extracellular surface electrodes [1, 2]. Despite the clear advantages of these devices, the information gathered by extracellular sensing is not exclusive to a single cell. Rather, it is an average over several cells located at the vicinity of the probe. For example, in the case of neuronal recording, extracellular probing does not provide the critical information about the DC state of a cell nor can it be used to easily identify the behavior of single cells.

Due to the limitations of extracellular sensing it is easy to recognize the enormous potential of intracellular probes for studying the physiology and the processes of *single, living* cells. In particular for understanding heterogeneous living cell populations that make up dynamic systems such as neural networks. The main challenge is to monitor single cell properties over time. Existing technologies for such probes are electrically or optically based and can provide valuable information such as neuronal potentials and chemical concentrations [3, 4, 5].

A major challenge in producing probes for intracellular sensing is the tip geometry. Intracellular probes must have extremely sharp tips (sub-micrometer dimensions) and they have to be long (>10 μm). These characteristics are necessary for effective bending and penetration of the flexible cell membrane (see Fig. 1).

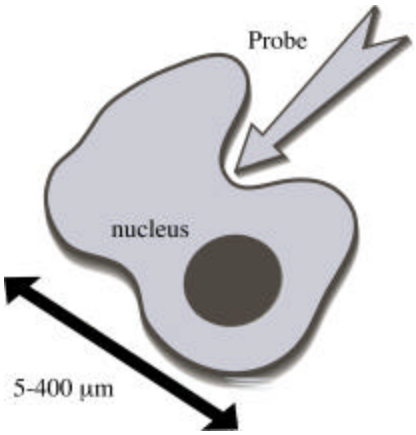


***Figure 1:*** *Schematic drawing of the bending of the cell membrane during insertion of a sharp probe. The probe cannot penetrate the cell membrane if it is too dull, or too short to compensate for the bending of the membrane.*

A standard technique to achieve tips with sub-micrometer dimension is by pulling heated glass capillaries [4] (see Fig. 2). These pulled micro-capillaries can be easily transformed into intracellular neuronal probes by filling them with an electrolyte and placing a Ag/AgCl electrode in the electrolyte. The glass walls (Fig. 2a) provide ionic insulation and ensure low leakage current.

Sharpening the edge of a fine insulated conducting wire can also create tips for neuronal recording [6]. In these

probes the signal is transferred from the tip through the wire while a dielectric material provides the insulation between the metal and the surrounding environment.

Similar probes are the micro-machined silicon needles [7]. Silicon as a structural material and micro-fabrication provide the option to extend the single needle fabrication process into complex structures such as large arrays with interconnects.

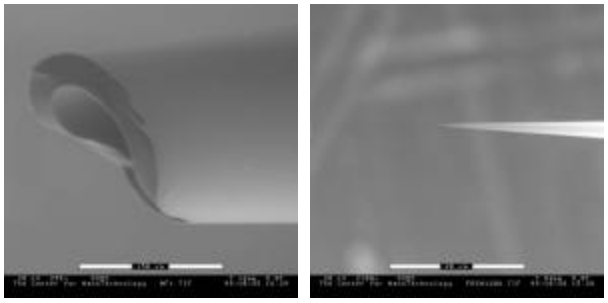

***Figure 2:*** *(a) ESEM image of a broken glass capillary. The glass provides superb insulation between the inner and the outer sides of the probe. The scale bar is 150 mm. (b) ESEM image of the tip of a pulled glass capillary. The scale bar is 20 mm. Similar tips are commonly used for intracellular neuronal recording.*

The focus of the current study is the realization of micro-machined silicon tips suited for intracellular probing. To ensure intracellular capabilities, we developed a procedure to produce single needles with geometry close to the conventional pulled glass capillaries mentioned above. We chose neuronal recording as a study case for the capabilities of our needles. To demonstrate the tip performance, we prepared and tested them as neuronal probes. We used two biological models: *Manduca sexta* (hawk moth) and *Tritonia diomedea* (a sea slug). The results, detailed below, confirm the qualities of the electrodes as extremely localized bio-sensors.

## FABRICATION

To fabricate needles suited for intracellular recording, we used highly conducting (n-type), 800 μm thick silicon wafers. Similar to the process in [7] we used a dicing saw to dice the wafer in two perpendicular directions to create arrays of tall pillars (70×70×350 μm). To sharpen the tips we used reactive ion etching (RIE) with $SF_6$. This is a robust, self-sharpening process, which we optimized in order to obtain long tips with high aspect ratio. The process lasts approximately 45 minutes and results in sharp, high aspect ratio needles (Fig. 3a).

In Fig. 3b we show the tip of a silicon needle, which we produced with the process mentioned above. Our process yields a probe geometry that is similar to the pulled glass electrodes commonly used in intracellular recording schemes (see Fig. 2b for comparison).

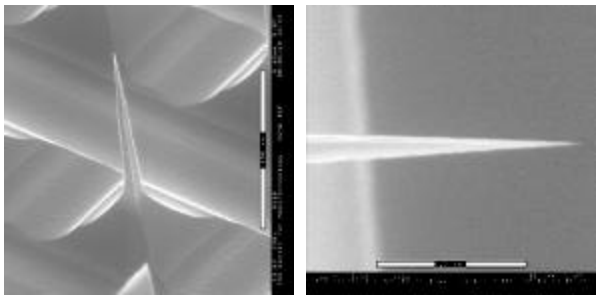

***Figure 3:*** *(a) A single needle after the RIE sharpening process. The scale bar is 150 mm. (b) A tip of a sharpened silicon needle. The needle is tilted approximately 45° with respect to the plane of the image. The scale bar is 25 mm.*

To produce separated needles, the wafer was bonded with photo-resist to another substrate and cuts to separate the parts were made prior to the sharpening process (Fig. 4). These cuts are approximately 500 μm apart. After the sharpening, the wafer was sputtered with Cr/Au (~70 nm) and with silicon nitride (~130 nm). Later, using an RIE $SF_6$ process, the nitride layer was slightly etched in order to expose the needle tips. Finally, we soaked the wafer in acetone and released the single needles.

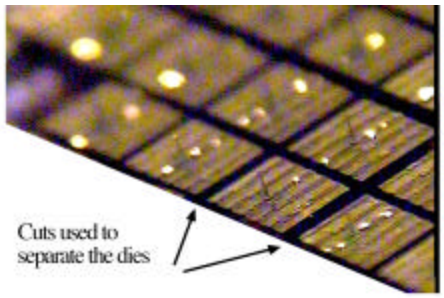


***Figure 4:*** *Optical microscope image of an array of diced electrodes. The electrodes are glued to a substrate with an adhesive (photoresist). By soaking the sample in acetone the dies can be separated and used as individual probes.*

## EXPERIMENTAL RESULTS

To test the performance of our electrodes we first used them in an extracellular preparation of *Manduca sexta* (hawk moth). A moth was anchored to a holder under a microscope and its lobula plate (the brain optic lobe) was exposed. A conducting wire was connected to the backside of a micro-machined needle device, and the needle was lowered into the lobula plate. A reference electrode was placed at a nearby position. In Fig. 4a we show a plot of the evoked extracellular potentials in the lobula plate. The data show well-defined single unit (one neuron) evoked potentials, which are likely a consequence of the narrow tip diameter of the MEMS probe.

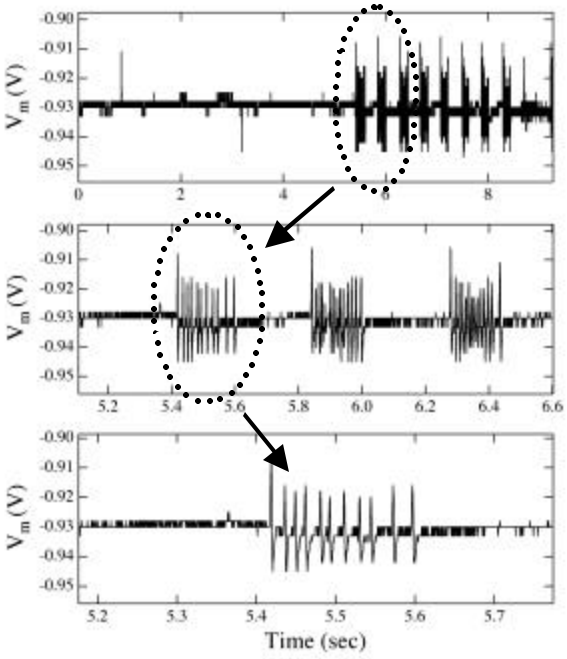


***Figure 4:*** *Evoked extracellular potentials in the lobula plate of Manduca sexta (hawk moth) plotted versus time (raw data).*

The extracellular capabilities, mentioned above, provide evidence for the quality of our sensors as localized electrical probes. To test the needles for intracellular applications we used the brain of *Tritonia diomedea* (a sea slug). Unlike the dry setup of the moth experiments, here the isolated brain was anchored in seawater under an optical microscope. A Ag/AgCl reference electrode was dipped in the seawater close to the brain. An insulated conducting wire was connected to the back of the micro-machined needle and the connection was insulated with varnish. At 100 Hz the electrode impedance was in the order of 1 MΩ.

Due to the enormous dimensions of the sea-slug brain cells (~400 μm) it is possible to monitor the penetration process and observe the accurate location of the probe as it approaches the cell. The micro-machined needles were mounted to a micro-manipulator and were slowly pressed against a cell membrane. Two effects were observed as the needles approached the cell: The measured background potential drifted, and the membrane bent. After moderate tapping on the measurement setup, spikes were observed. This is likely due to cell membrane penetration. The recorded data is shown in Fig 5. Similar tests with dull electrodes (tip sizes ~ 5 μm) did not result in any spiked signal.

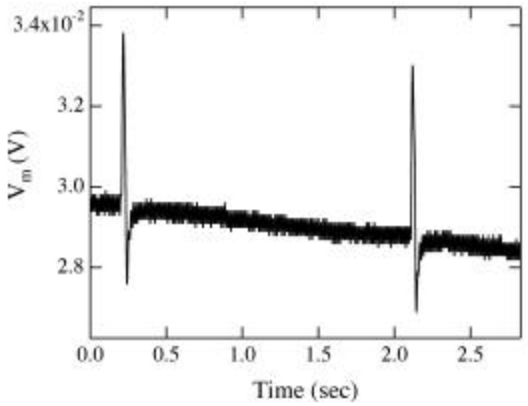


***Figure 5:*** *Spontaneous intracellular potentials in a neuron in the brain of Tritonia diomedea (a sea slug) plotted versus time. The positioning of the probe was controlled via micro-manipulators and an optical microscope.*

The measured noise level seen in Fig. 5 is due to background noise (60 Hz) and possibly due to insufficient insulation. This issue is discussed in more detail later in the text. Bio-fouling (the affinity of proteins to adhere to synthetic surfaces [8]) may also contribute to the noise and the instability, which was observed over two separated tests of one hour of recording. An approach to improve the electrode bio-compatibility is mentioned in the next section. Finally, a damaged membrane is likely to contribute to the noise levels as well as to the relatively low and slow-varying signal seen in Fig. 5. Future work will include improved insertion techniques to avoid damaging the cell membrane.

To conclude the results presented so far, the similarity between the geometry of the silicon needles and the pulled glass capillaries (see Figs. 4 and 2b) support the potential of our process to produce intracellular silicon based needles. Further, the data in Figs. 4 and 5 hint at

the exciting possibilities for full-fledged neurobiological experiments using silicon based electrodes.

## CURRENT AND FUTURE WORK

The current design of our devices allows a convenient handling by using the large base (Fig. 3), to hold and manipulate the needles. During the measurements, however, when the needles are soaked in a conducting medium (i.e. seawater or blood) the base acts as a large capacitor (impedance of ~10 MΩ at 100 Hz). This capacitor is in parallel to the active sensor (the tip of the needle) and can cut off valuable data. A better design should take this into account by limiting the dimensions of the base.

As a first step to improve our process, the dicing saw will be replaced with a deep reactive ion etching (DRIE) process. This will allow a versatile design of needle arrays. Future work will also focus on a versatile connectivity scheme. Preliminary work suggests that polyimide can be used as a convenient structural flexible connecting material. The qualities of the polyimide as a good ionic insulator can be employed to protect metallic lines, which will be used to connect the electrodes and to build large needle arrays. Finally, to enhance the electrode bio-compatibility a non-fouling coating will be deposited. Such a coating was recently tested for bio-MEMS applications and was verified to have good adhesion to silicon, nitride and gold [9]. Also, it was found that this coating has good ionic conductivity.

## SUMMARY

In summary, we presented a technique to produce sub-micrometer sharp, high aspect ratio, silicon needles. With the refined geometry we were able to obtain high quality *in vivo* extracellular recordings. Moreover, we demonstrated a first evidence for cell penetration and recording with silicon needles inside a cell. With the advances in bio-MEMS along with the techniques we discussed here the long term goals of our research is to build stand-alone implantable sensing units made of probes, amplifiers, and memory components, with the specific goal to allow intracellular recording from freely behaving animals.

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