1 INTRODUCTION

Contemporary tools for studying neuronal signaling and information processing include micropipette and micromachined extracellular probe and imaging at the cellular and anatomical levels. Intracellular recording is preferred to extracellular recording since the signal-to-noise ratio is much better.

Experiments with intracellular glaspipettes filled with KCl are done in situ which means that the live laboratory animal has to be fixed relatively to the equipment during the tests.

So far many of these experiments have been done in the sea slug "Tritonia diomedea" due to its relatively simple structure of the brain. It consists of approximately 10,000 brain cells (neurons) whereby the diameters of the cell bodies range from the same size as in vertebrates ($30\mu m$) up to $500\mu m$.

Recording intracellularly in live, freely behaving animals would represent substantial progress in neurobiological research. To achieve this goal, MEMS-based techniques may be an auspicious approach.

These techniques make the fabrication of microsensors on microchips possible. By using contemporary surgical procedures these microchips could be implanted into the brain of Tritonia diomedea. Another advantage of this sea slug are the reidentifiable brain cells. This means that certain brain cells for specific functions can be found again at the same place in all animals of this species. When recording from these cells in different animals, the data can be directly compared.

Another advantage of a small recording device is that several of them can be implanted to provide information from different parts of the brain simultaneously. This might also help to understand the communication between neurons. Since the animals are allowed to move freely, a long-term observation possibility should be also provided. It might be achieved by a memory chip connected to a control chip with integrated data compression algorithms.

Currently, the basic architecture of the microsystem consists of a sensor on a substrate, a control chip, a memory chip and power supply. The changes of the potential of the brain cells are transduced by the sensor into electrical signals which are compressed by the control chip and stored in the memory chip.

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Hereby the advantages of IC fabrication like mass fabrication and use of the mechanical properties of silicon can be used for the production of the single components of the system. In such a system the computational power of silicon electronics is harnessed to the real-time study of intracellular neuronal information processing. Thus the gap between silicon and neurons would be reduced. In the far future these results could help to build computers which are based on the functional principles of brains.

2 NEUROBIOLOGICAL BASICS

In this chapter the neurobiological basics are developed as far as it is necessary for the design of a recording device. After the introduction to the membrane structure an explanation for the resting and action potentials will be given. Furthermore the electrical properties of cells will be examined.

2.1 Membrane Structure



The membrane consists of lipids and proteins. Phospholipids are the most abundant lipids in most membranes. They possess both a hydrophilic head and a hydrophobic tail. Charged phosphates build the head whereas fatty acids construct the tail.

In an aqueous environment these molecules spontaneously orient themselves to form a double layer. This means that the fatty acid ends of two layers come together and the hydrophilic heads face towards the aqueous environment (see Fig. 2.2) [1].

This phospholipid bilayer can fold and thus make a shell with water both on the outside and the inside. The permeability of the lipid bilayer is determined by the nonpolar character of the hydrophobic core. Thus the transport of ions and bipolar molecules, which are hydrophilic, is impeded. Hydrophobic molecules such as oxygen and hydrocarbons can dissolve in the membrane and cross it easily. Small polar but uncharged molecules such as water and carbon dioxide can also pass between the lipids of the membrane. Large uncharged molecules like glucose pass through the membrane only with big difficulties. In Fig. 2.3 a survey of the different permeabilities is given.

Since the main bonding principle is hydrophobic between the fatty acids, the rigidity of the membrane composed only of lipids would be quite weak. Lateral movements of the phospholipids would be possible without any further support molecules. That is why cholesterol molecules are necessary. They are immersed in the membrane and provide more stiffness by hindering the sliding of the phospholipids.

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Large proteins can only move slowly laterally and thus improve further the rigidity of the membrane [2]. They also consist of two hydrophilic heads and a hydrophobic body so that they can be built into the bilayer. Besides improving the rigidity, proteins fulfill several other tasks in a membrane. Examples are:

- Enzymes: catalyze reactions on the surface of the membrane
- Transport: some proteins build hydrophilic channels for certain ions
- Receptors: bind neurotransmitters and hormones
- Energy: some proteins hydrolyze ATP as an energy source to actively pump substances across the membrane (see chapter 2.2)



2.2 Resting Potential

As mentioned in chapter 2.1 there exist proteins which act as a pump. One pump is called the Na^+/K^+ -ATPase which transports in each cycle 2 K⁺ ions from the outside of the cell to the inside and 3 Na⁺ ions from the inside to the outside. The energy for this transport is generated by the hydrolyzation of ATP (Adenosine triphosphate) [2].



Due to the Na^+/K^+ pump there exist concentration differences between inside and outside of the cell. Table 2.1 shows rough values for animal neurons (in [mM]; A stands for anions):

	Inside	Outside
K+	150	5
Na⁺	15	150
Cl-	10	120
A-	100	10

Table 2.1: Ion concentrations [6]

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Proteins can also act as hydrophilic ion channels (see Fig 2.6). Each channel is only permeable for a certain kind of ions. During the resting state of the cell the permeability of the membrane is much higher for K^+ ions than for Na⁺ ions. Thus there is a tendency for the K^+ ions to diffuse out of the cell



down the concentration gradient. This implies that the potential in the cell must be negative relative to the outside due to the outflux of positively charged ions. This process will last until there is an equilibrium between the electrical forces based on attraction forces between charged ions of different sign and the chemical forces due to concentration gradients. The electrical work can be determined by the following equation [1]:

(2.1)



The chemical work along a concentration gradient is given by:

 ΔG=R·T·ln([cation]_i/[cation]_o)
 (2.2)

 R: gas constant; R=8.3 J/(mol·K)
 T: temperature in Kelvin

 i: inside cell
 0: outside cell

In equilibrium these two works must be the same. Equating delivers the Nernst equation:

$$E_{M} = \frac{R \cdot T}{z \cdot F} \cdot \ln \frac{[\text{cation}]_{o}}{[\text{cation}]_{i}}$$
(2.3)

This equation is only valid for one specific kind of ion. Furthermore it does not take the different permeabilities for different ions into consideration. Goldman, Hodgkin and Katz developed a new equation during the 1940s which interprets permeabilities as conductances g_i:

$$E_{M} = \frac{g_{K} \cdot E_{K} + g_{Na} \cdot E_{Na} + g_{Cl} \cdot E_{Cl}}{g_{K} + g_{Na} + g_{Cl}}$$
(2.4)

Plugging in the values of Table 2.1 yields E_M =-70 mV. This is slightly higher than the result for K⁺ alone ($E_M(K^+)$ =-80 mV) since this equation also respects the influx of Na⁺ ions down its concentration gradient into the cell.

2.3 Action Potential

As described in the previous chapter the conductivity for K^+ ions is much higher than that for Na⁺ ions because of more open specific ion channels. Neurons are stimulated by raising the membrane potential in certain areas. If the potential exceeds a certain threshold the K^+ channels are closed and some Na⁺ channels are opened. The results are: the outflux of K^+ ions is prevented and the influx of Na⁺ ions is raised. This in turn makes the potential of the cell



relative to the outside less negative and because of that more so-called voltage gated Na⁺ channels



are opened. Then the influx of sodium ions increases again and the potential raises even more (depolarization). This process is called the "Hodgkin cycle" (see Fig. 2.8) and this phase of the action potential "depolarizing phase". The potential that is finally reached is approximately $E_M = +30$ mV. It is lower than the theoretically possible $E_{na}=+60$ mV. The reason for this is that the voltage gated sodium channels inactivated after are approximately 1ms. The conductivity for Na⁺ ions is thus reduced to its original value. Simultaneously voltage gated K⁺ ion channels are opened. This causes an outflux of K⁺ ions and thus a reduction of the potential of the cell (hyperpolarization). The period is called the repolarizing phase. Since the potassium

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channels are slower than the sodium channels they still remain opened even when the potential reaches its resting level again. They close shortly after that thus causing an "undershoot". During the undershoot the cell is insensitive to depolarization and therefore also called the "refractory period". After another millisecond or two the resting state is then restored. The whole event with the four states (depolarization, hyperpolarization, undershoot, resting state) is an all-or-none-event which means that once the threshold is exceeded the complete sequence is executed. Furthermore it consumes only few energy since the coordinated actions of the ion channels induce only a low flux of ions [2]. The action potential travels along the length of neurites, allowing neurons to communicate with each other over long distances.



2.4 Electrical Properties of the Membrane

The membrane is a part of the electrical circuit while measuring the intracellular potential and thus has to be taken into account. The membrane channels have different permeabilities for different ions and these permeabilities also depend on whether there is an action potential or not. With patch clamp techniques (extracellular glass capillaries) it is possible to observe single ion channels. The obtained data leads to the conclusion that the resistance varies from 5 G Ω to 500 G Ω depending on the channel type and tissue. The overall membrane resistance then ranges from 1 M Ω ·µm² to 100 G Ω ·µm² [1].

Furthermore the membrane forms an insulating layer between two conducting solutions thus making a capacitor. The capacitance can be calculated by:

$$C = \frac{\acute{\mathbf{e}}_{r} \cdot \acute{\mathbf{e}}_{0} \cdot A}{d}$$
(2.5)
 ε_{r} : relative permeability, here ε_{r} =6 [3]

 $\epsilon_0\!\!:$ permeability of free space ($\epsilon_0\!\!=\!\!8.85\!\cdot\!10^{\text{-12}}\,C\!\cdot\!V^{\text{-1}}\!\cdot\!m^{\text{-1}})$

d: distance between the two layers (here d=8 nm)

A: area



Changes of the permeability of ion channels during action potentials should not significantly alter the

capacitance since the channel density over the membrane is relatively low. Thus solving equation (2.5) results in a value of approximately c=.01 pF/ μ m².

The simplest equivalent circuit is a parallel RC circuit with resistances representing the different ion channels (see Fig. 2.10) [1].

2.5 Structure of the Brain of "Tritonia diomedea"

In this chapter a short overview of the structure of the brain of Tritonia diomedea will be given as far as it is necessary for the design of the probe. The actual brain cells are protected by the perineurium (a special membrane layer), and the glia cells which are arranged between the perineurium and the brain cells (see Fig. 2.11). A probe tip has to penetrate these two layers and the cell membrane. The brain consists of 6 ganglia which are congregations of brain cells. Nerves are bundles of neurites.



3 CONCEPT

In order not to forget or overlook possible variants the finding and choosing of ideas is done systematically. First of all the definition of goals has to be translated into a list of technical requirements. After finding solutions for these requirements these ideas have to be assessed and then chosen accordingly.

3.1 Targets

The definition of goals describes the targets which should be reached with this project:

- Insights about the habits of the nudibranch Tritonia diomedea
- Insights about the function of neural system of animals
- Interface between biology and silicon electronics (close the gap between neurons and computers)

3.2 Demands for the Probe

During the first meetings with the other scientists involved in the project the exact requirements for the probe were determined. They were separated into "musts" (requirements that have to be fulfilled), "preferred" (requirements that should be achieved) and "wishes" (features that might be implemented if possible). This led to the following lists:

Musts:

- Storage signals from Tritonia neurons either RAM either inside or outside the animal
- Signals must be neurobiologically relevant
- Able to survive in sea water

- Maximum dimensions (=dimensions of the brain) are 1cm long, 5mm wide and 2mm deep, ideal 1mm²x500µm
- Fixed relatively to brain cells (the smaller the size the easier; should be completely covered by glue; movements may cause damage either to the cell or the probe)
- Get through intervening biomaterial down to the neurons (diameter of tip must be ${<}1\,\mu\text{m})$
- Biocompatible
- Must not damage cells significantly
- Lifetime several hours (24h+)
- Connection to control-chip

Preferred:

- Information about the intracellular voltage potential
- Stimulation
- Signals from as many neurons as possible (at least 4)
- Means to see where probe/probe array is put
- Option to go extracellular

Wishes:

- Robust
- Robust reference electrode (movements of the probe cause changes in the resistance between the two electrodes since the distance is longer or shorter; $R=\rho\cdot l/A$
- Injection of dye

3.3 Choice of Functional Principle

Each time when a decision has to be made, e.g. the choice of a functional principle for a functional unit, this decision should be done systematically. This means that criteria for the decision have to be found and weighted. The first choice that had to be done was to choose a proper principle for measuring the signals in the brain cells. In several brain storming meetings with the project members the following functional principles were found:

- Magnetism (changes in magnetic field)
- Changes of weight (changes of resonance frequency)
- Optical means (changes in refraction)
- Electrode
- Capacitance (changes between two capacitor plates)
- Pressure (membrane might show buckling)
- Chemistry (e.g. ion selective probe)
- Changes in membrane (amount of ion channels)
- Voltage clamps
- Temperature
- Electrostatic influence

Since the probes are developed for neurobiologists the weight of the different criteria was done by Russell Wyeth from the Department of Zoology. Weights were only made for "Preferred" and "Wishes" since a "Must" has to be fulfilled in any case. A principle not fulfilling a "Must" will not be considered in the future (thus the entries in the form are empty). Some more annotations are necessary to explain some of the weights. "Intracellular potential" is not a "Must" because brain signals could also be won extracellularly but intracellular recording provides clearer signals (they are not disturbed by signals from other cells). So their weight is 10. Some of the criteria like "Injection of dye" and "Stable reference electrode" refer more to the long term possibilities of the project and are not necessary for the design of a first prototype. That is the reason why they get a low weight. Furthermore the highest possible weight for "Preferred" was set to 10 whereas the according weight for "Wishes" was set to 5 to show the difference in importance. Table 3.1 shows all the weights.

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Criteria	Weight				
Intracellular potential	10				
Stimulation	8				
Many neurons	7				
Location of probe known	5				
Robust	3				
Injection of dye	2				
Option extracellular	5				
Stable reference electrode	2				

 Table 3.1: Weight of criteria

These weights were put into a form to get a clear and comprehensible presentation of the decision process. After that form had been made a meeting took place where the basic functional principle had to be found. The principle "Electrode" was finally chosen because it offers the best prospects for intracellular recording.



3.4 Functional Structure

In order not to forget some important parts of the project a functional structure of the probe had to be found. For each substructure ideas to fulfill its specific task have to be found and then evaluated. Fig. 3.2 shows the functional structure (Dotted means optional. These options were not considered in this thesis).



3.4.1 Choice of Variants for "Tip Unit"

First of all criteria and weights for a decision had to be found. This led to the following table:

Criteria	Weight
Biocompatible	9
Dimensions	8
Fabrication at WTC possible	5
Get to neuron	10
Intracellular signals	10
Insulated against outside	9
Robust	7
Minimize damage to cell	7

 Table 3.2: Weight of criteria

Again, some comments are necessary. Since from now on everything is based on the principle "Electrode" the intracellular option is the one that will be continued. That is the reason why only the upper part of the tip may be conductive. Otherwise also signals from outside the cell could be recorded. This is considered with the criteria "Insulated against outside". It is supposed that the same insulation process can be used for each of the variants. During another brain storming meeting the following ideas for the fabrication of the "Tip Unit" were found:

- Anisotropic wet etch of Si with KOH
- LIGA fabricated needles
- Microneedles [5] (silicon microneedles made by "Reactive Ion Etching", RIE)
- E-beam induced microneedles [6] (microneedles grown in a gas atmosphere by applying energy in an e-beam)
- Microneedle in plane [7] (tip is created by a predictable fracture of a microbeam)
- Melting + Pulling (blunt plastic needles are heated until viscous and then pulled)
- SiO₂ cone filled with metal (for the fabrication of the needles see [8])

These ideas were evaluated again in a form according to the criteria above (see Fig. 3.3). The result was that the fabrication of microneedles with a process proposed by [5] was chosen.

			138			 }						7
Variants	Ŕ	ACOTIO	MUE C	abroat c		ICO II		SON N	winter connert	4	5	
Anisotropic wet etching of Si	+	?	+	?	+	+	+	-	Geometry restricted by crystal planes	40		
LIGA	+	+	-	?	+	+	+	?	No papers about microneedles available	43		
Microneedle	+	+	+	+	+	+	?	+	See [5]	58		
E-beam induced needle growth	+	-	-	-	+	+	?	+	See [6]	35		
Microneedle in plane/breaking	+	+	+	?	+	+	+	?	See [7]	48		
Melting + pulling	+	+	+	+	+	+	?	+	Problem: manual fabrication	53		
SiO ₂ cone filled with metal	+	?	?	?	+	+	?	?	No experience	33		
Weight	9	8	5	10	10	9	7	7				
Weight: 1-10 resp. 1-5 for Wishes (5 resp. 10 best, 1 worst or least) Signs: ?: lack of information, !: check requirements												
Date: 02/26/99												
Böhringer, Lang												
• Fig.3.3: Choice of variants for "Tip Unit"												

3.4.2 Choice of Variants for "Actuator Unit"

The procedure for choosing appropriate ideas for the movement of the tip into the cell is the same as in the preceding chapters. Soon one problem was found: no data about the necessary force to get into a neuron is available. So far all the electrodes were driven manually and supported by the "buzz", an AC voltage applied to a KCl electrode [9]. This probably leads to changing attraction and repelling forces on the membrane which finally can be easily penetrated. The search for appropriate functional principles soon showed that there is another fundamental problem: most of the actuator materials cannot be used in a fluidic environment (e.g. pneumatic, ...). Thus only three basic ideas could be found:

- Manual (by hand or knocking on the backside of the probe)
- Piezo (piezoelectric actuator)
- Thermal stress (Residual stresses in a cantilever which is chucked and then released)

Table 3.3 shows the criteria and their weight.

Criteria	Weight
Impulse high enough	10
Biocompatible	9
Fabrication at WTC	5
Dimensions (mustn't be too large)	8
Sensor for location of tip	2

 Table 3.3: Criteria and weights for choosing "Actuator Unit"

Again, the ideas and this table were put into a form and then a decision was made. Fig. 3.4 shows how the decision was made. So far the probes have been actuated manually so this variant was chosen due to the experience that has been collected. At this point all the important and basic decisions have been made. An electrode driven manually, shaped like a microneedle and fabricated by using a RIE process is the approach towards the goal of the project.



3.5 Geometric Dimensions

At this point some assumptions about the probable geometric properties of the probe can be made. They are only a rough estimation but they will be useful for a first calculation of the mechanical and electrical properties of the probe tip. These calculations are not supposed to be exact but they will show the order of magnitude of the properties of the probe tip. Fig. 3.5 shows the overall dimensions that can be expected after the choice of variants. They will be necessary for the calculation of mechanical stresses. A closer look at the exposed actual electrode is shown in Fig. 3.6 and will be used for the calculation of the electrode impedance. Since many electrode properties are dependent on the exposed metal area of the probe a short calculation is given here. It is considered to be a cone since the tip will not be an exact point but somewhat blunt. With the notation in Fig. 3.6 the following calculation steps have to be done. First of all the line M is calculated:

$$M = \sqrt{\left(\frac{D - d_{iip}}{2}\right)^2 + h^2}$$
(3.1)
= $\sqrt{\left(\frac{3\ddot{U} \ m - 0.5\ddot{U} \ m}{2}\right)^2 + (10\ddot{U} \ m)^2}$
= 10.1 mm

which is used to calculate the surface area:

$$A_{M} = \frac{\dot{\mathbf{a}} \cdot M}{2} \cdot (D + d_{iip})$$

$$= \frac{\dot{\mathbf{a}} \cdot 10.1 \ddot{\mathbf{U}} m}{2} \cdot (3 \ddot{\mathbf{U}} m + 0.5 \ddot{\mathbf{U}} m) = 55.5 \ddot{\mathbf{U}} m^{2}$$
(3.2)

The area of the blunt tip is:

$$A_{tip} = \pi \cdot r^2 = \pi \cdot (d_{tip}/2)^2 = \pi \cdot (0.25 \ \mu m)^2 = 0.2 \ \mu m^2$$
(3.3)

The total electrode area is consequently:

$$A_{tot} = A_M + A_{tip} = 55.7 \ \mu m^2$$
 (3.4)





4 BASICS OF ELECTRODES

In this chapter a brief overview of the theory of electrodes will be given. Topics will be the solidelectrolyte interface, the resistance of the interconnects and the sources of noise and other signal attenuations. Since all these issues are very complex this chapter will give only the basic understanding which is necessary for the design of the electrodes. This discussion here follows closely an overview given in in [3]. Other good introductions were given in [10, 11, 12, 13].

4.1 Solid-Electrolyte Interface

Between electric conduction in solutions and in solids there is one basic difference: almost always in liquids it is based on dissolved ions whereas in solids it is based on electrons. If there is a closed circuit with both solids and liquid parts then somewhere the transformation from ionic to electronic conduction has to take place. This happens at the surface of an electrode.

4.1.1 Processes on the Surface of an Electrode

Once a metal electrode is immersed in an electrolyte the following equilibrium is established:

$$M \leftrightarrow M^{\scriptscriptstyle +} + e^{\scriptscriptstyle -}$$

While the processes are often very complex a possible explanation for this phenomenon has been proposed in [14]. The author presumes that the reactions on the surface of an electrode are different from those in the bulk. In the bulk the forces on an ion are symmetrical since it has the same number of neighbors in every direction. On the surface however it is surrounded only by half of the number of ions thus leading to asymmetrical forces. Furthermore it can be surrounded on one side by polar molecules in the electrolyte. Once this ion is attracted by polar molecules these forces will gradually become larger than the forces in the bulk acting on this ion and finally the ion will go into solution surrounded by a hydrate layer. It leaves a negative charge in the bulk so that the interface as a whole remains electrically neutral. But as more and more ions are dissolved the bulk becomes more and more negative and thus makes it more difficult for positive ions to leave the bulk. The process comes

to a dynamic equilibrium which means that the net charge transfer is zero but forward and reverse reaction still take place. Fig. 4.1 shows the electrostatic forces in an ion crystal but they can also be interpreted as forces between ion and electron gas in a metal.



4.1.2 Space Charge Layer

As described in the previous chapter a charge separation at the electrode/electrolyte interface takes place reaching a dynamic equilibrium. These reactions lead to a space charge layer close to the electrode surface. The center of the ions that have the opposite charge as the electrode are in a layer called the "Outer Helmholtz Plane" (OHP). From this maximum point their concentration declines to the bulk concentration far away from the influence of the electrode. This area is called the diffuse space charge layer. The "Inner Helmholtz Plane" is built by water molecules like previously mentioned. Due to their polarity they accumulate on the surface of the electrode.



4.1.3 Helmholtz Capacitance

As shown in the previous chapter there are basically two charged layers separated by an insulator. The two layers are the OHP and the surface of the electrode whereas the IHP acts as the dielectric. Thus a parallel-plate capacitor is obtained which can be calculated by the wellknown formula:

$$C_{H} = \varepsilon_{0} \cdot \varepsilon_{r} \cdot A/d$$
(4.1)

$$\varepsilon_{0}: \text{ dielectric permittivity of free space (8.85 \cdot 10^{-12} \text{ F/m})}$$

$$\varepsilon_{r}: \text{ relative dielectric permittivity (6 in the interface [3])}$$

A: area of plates
d: distance between areas

Assuming an OHP distance of 5 Å, this yields a specific value of $c_H=0.11$ F/m². Experimental data is not totally consistent with this model. The main difference is that the actual capacitance is potential

dependent. When the potential applied to an electrode is increased, ions try to pack closer to the surface and the diffuse space charge layer will shrink in thickness. Applying c_H to the actual problem with A_M =55.7 µm² leads to:

$$C_{\rm H} = 0.11 \ \text{F/m}^2 \cdot A_{\rm M} = 0.11 \ \text{pF/\mu} \text{m}^2 \cdot 55.7 \ \mu \text{m}^2 = 6.1 \ \text{pF}$$
(4.2)

4.1.4 Gouy-Chapman Model

To describe the actual situation better a new model was introduced by Gouy and Chapman. Their model predicts a potential profile in the space charge region which is given by

$$tanh(z \cdot q \cdot V/4 \cdot k \cdot T)/tanh(z \cdot q \cdot V_0/4 \cdot k \cdot T) = tanh(z \cdot V/4 \cdot V_t)/tanh(z \cdot V_0/4 \cdot V_t)$$
$$= exp(-x/L_D)$$
(4.3)

with

$$L_{D} = \sqrt{\acute{\mathbf{e}}_{0} \cdot \acute{\mathbf{e}}_{r} \cdot V_{t} / 2 \cdot n^{0} \cdot z^{2} \cdot q}$$

$$\tag{4.4}$$

V₀: potential at the electrode V: potential at distance x from electrode k: Boltzmann's constant (1.38·10⁻²³ J/K) T: temperature in K q: charge of an electron (1.6·10⁻¹⁹ C) n⁰: bulk number concentration of ion (mol/1 · Avogadro's number, 6.02·10²³ mol⁻¹) z: charge of ion V_t=k·T/q, at 25°C V_t=26 mV L_p: Debye length (a small perturbation of potential decays "e"-fold over this length)

According to this model the voltage drop through the region near the electrode is approximately exponential. Furthermore for small electrode potentials ($V_0 \le 50 \text{ mV}$) the equation (4.3) can be further simplified since then:

$$\tanh(z \cdot V/4 \cdot V_t) \approx z \cdot V/4 \cdot V_t \qquad \text{if } z \cdot V/4 \cdot V_t \leq 0.5 \qquad (4.5)$$

thus the following can be obtained:

$$V(x) = V_0 \cdot exp(-x/L_D)$$

$$(4.6)$$

For physiological saline, which is a 0.9% NaCl solution, C^* is 0.154 mol/l and z is 1. Using furthermore T=298 K and ϵ_r =6 this yields the following value for L_D :

$$L_{D} = \sqrt{8.85 \cdot 10^{B12} \frac{F}{m}} \cdot 6 \cdot 0.026 V / 2 \cdot C^{*} \cdot 6.02 \cdot 10^{23} mol^{B1} \cdot 1 \cdot 1.6 \cdot 10^{B19} C}$$
(4.7)
=2.15 \cdot 10^{-10} m
C^{*}: bulk concentration (mol/l)

By applying Gauss's law to the charge distribution near the electrode the value of the capacitance of the diffuse space charge layer can be derived:

$$c_{\rm D} = \varepsilon_0 \cdot \varepsilon_r \cdot \cosh(z \cdot V_0 / 2 \cdot V_t) / L_{\rm D} \quad \text{in } F/m^2$$
(4.8)

If we consider a V_0 value of -70 mV (resting potential) in physiological saline then:

$$c_{\rm D} = 0.5 \ {\rm pF}/{\rm \mu m^2}$$
 (4.9)

Multiplying with A_{tot} yields the actual value of C_D in this application:

$$C_{\rm D} = c_{\rm D} \cdot A_{\rm tot} = 0.5 \text{ pF}/\mu m^2 \cdot 55.7 \ \mu m^2 = 28.2 \text{ pF}$$
(4.10)

4.1.5 Stern Model

Neither the Helmholtz model nor the Gouy-Chapman model explain accurately enough the capacitive properties of the charge distribution near the electrode. The Helmholtz model does not vary with the applied voltage whereas the values obtained with the Gouy-Chapman model vary too strongly with the applied voltage. This contradiction was resolved by Stern. He proposed a series of a Helmholtz capacitor and a Gouy-Chapman capacitor, which results in the actual conditions near the electrode being described best. We have a sheath of ions attached to the electrode and a diffuse charge layer (see Fig. 4.2). The Stern or interface capacitance is consequently:

$$\frac{1}{C_{I}} = \frac{1}{C_{H}} + \frac{1}{C_{D}}$$
(4.11)

Here:

$$\frac{1}{C_{I}} = \frac{1}{6.1 \ pF} + \frac{1}{28.2 \ pF} = 0.2 \frac{1}{pF}$$

$$C_{I} = 5 \ pF$$
(4.12)

4.1.6 Charge Transfer Resistance

While the capacitive character of the electrode/electrolyte interface is mainly of interest during recording, the resistive properties have to be taken into consideration for stimulation. The resistive part is in parallel with the capacitive path. To move an ion in additional to the ions of the dynamic equilibrium into or out of an electrode requires a higher potential than the potential of steady-state. This potential is called the overpotential η . It is defined as follows:

$$\eta = V - V_0 \tag{4.13}$$

It is composed by four independent overpotentials:

$$\eta = \eta_t + \eta_d + \eta_r + \eta_c \tag{4.14}$$

 $\eta_t\!\!:$ overpotential due to charge transfer through the double layer

 $\eta_{\text{d}}\!\!:$ overpotential due to diffusion of reactants to and from the electrode

 $\eta_{r}\!\!:$ overpotential due to chemical reactions at the electrode

 $\eta_{\rm c}\!\!:$ overpotential due to crystallization actions on the electrode surface

In the present discussion the overpotential η_t is the most important one since it occurs for any current flow. This overpotential is higher than the others under the conditions in this project. The overpotential η_d becomes more important when limited rates of supply of reactant ions from the bulk solution start limiting the current. This usually happens at higher frequencies since then the ions can no longer follow the applied field due to their inertia. The remaining two overpotentials are not very significant in biological applications and thus will not be considered any more in this discussion.

Overpotential η_t :

In the dynamic equilibrium the forward and reverse reaction are equal and of opposite sign. The absolute value of this current for a given surface area is called the exchange current density, J_0 . When additional current is passed through the electrode the overpotential η_t is determined by this current with respect to J_0 . If this current is low the electrode is not driven far from equilibrium and it will act like a linear resistive element. The value of η_t can be determined in relation to the current density by the Butler-Volmer equation:

$$J=J_{0}\cdot[e^{[(1-\beta)\cdot z\cdot\eta_{t}^{/V}]}-e^{(-\beta\cdot z\cdot\eta_{t}^{/V})}]$$

$$\beta: \text{ symmetry factor}$$

$$(4.15)$$

The symmetry factor indicates differences in the electronation and deelectronation reactions and hence determines differences in the negative and positive current range of the I-V characteristics. Low J_0 currents require large voltages to produce appreciable currents. This is undesirable due to

electrochemical reactions during stimulation.

In the case of a nonrectifying system (negative voltage I- η_t curve is the inverted mirror image of the positive curve) the symmetry factor equals 0.5. Hence equation (4.10) becomes

$$J=2\cdot J_0 \cdot \sinh(z \cdot \eta_t / 2 \cdot V_t) \tag{4.16}$$

which leads to an incremental resistance of

$$\mathbf{R}_{t} = \mathbf{\eta}_{t} / \mathbf{\eta}_{t} = \mathbf{V}_{t} / [(\mathbf{J}_{0} \cdot \mathbf{z}) \cdot [\cosh(\mathbf{z} \cdot \mathbf{\eta}_{t} / 2 \cdot \mathbf{V}_{t})]]$$

$$(4.17)$$

This can be further simplified by two useful approximations. The low-field approximation (which is the case for recording) is a simple mathematical one for trigonometric functions:

$$\sinh(z \cdot \eta_t / 2 \cdot V_t) \mathbf{Z} z \cdot \eta_t / 2 \cdot V_t \qquad \text{for } z \cdot \eta_t / 2 \cdot V_t <<1 \qquad (4.18)$$

which gives

$$J=J_0\cdot(z\cdot\eta_t/V_t) \tag{4.19}$$

Another assumption made is:

$$z \cdot \eta_t / V_t < 1/5 \tag{4.20}$$

Hereby the limiting value for η_t can be determined by

$$\eta_t < 0.005/z$$
 (4.21)

Thus within the linear limits the resistance due to charge transfer can be obtained:

$$R_{t} = \gamma_{t} / J = V_{t} / (J_{0} \cdot z)$$
(4.22)

The high-field approximation which is necessary for stimulation applications is (without derivation):

$$J = J_0 \cdot e^{(z \cdot \eta_t^{/2 \cdot V})}$$

$$\tag{4.23}$$

In this work the focus is on recording applications so that only the charge-transfer resistance for lowsignal responses will be calculated. First of all V_t has to be calculated. Since it will only be a rough estimation the calculation will be done for a temperature of 25°C although the temperature in Tritonia diomedea itself is dependent on the water temperature. The other necessary constants for this calculations are given in (4.4) so that:

$$V_{t} = \frac{k \cdot T}{q} = \frac{1.38 \cdot 10^{-23} \cdot 298 K}{1.6 \cdot 10^{-19} C} = 26 \, mV \tag{4.24}$$

As can be seen in Table 4.1 the exchange current density for gold in buffered saline is $J_0=3.98 \times 10^{-6}$ A/cm². Put into equation (4.22) this yields for univalent ions

$$R_{t} = \frac{0.026 V}{3.98 \cdot 10^{-6} \frac{A}{cm^{2}}} = 6.5 \cdot 10^{3} \quad \mathbf{D} \cdot cm^{2} = 6.5 \cdot 10^{11} \quad \mathbf{D} \cdot \mathbf{U} m^{2}$$
(4.25)

for the given area A_M:

$$R_{t} = R_{t} / A_{M} = 6.5 \cdot 10^{11} \,\Omega \cdot \mu m^{2} / 55.7 \,\mu m^{2} = 1.2 \cdot 10^{10} \,\Omega$$
(4.26)

4.1.7 Electrode Polarization

Electrochemists describe with the term polarization the removal of an electrode from its equilibrium under an applied steady-state potential. For neurobiological application a nonpolarizable electrode is desirable. This requires that J_0 is infinite and every current due to neurobiological events would be small in relation to the exchange current density and thus η_t would be small. Thus, even small neurobiological events which are usually in the mV range could be recorded. Recording is not possible when η_t is higher than the signal to be recorded. Fig. 4.3 shows this concept. The goal for the electrode material is thus to have a very high J_0 . Among the noble metals Gold has the lowest J_0 whereas Pt has the highest one. J_0 can be improved by electrodeposition of Pt on a metal [1, 10, 13, 15, 16]. This results in a very spongy layer of platinum on the electrode ("platinum black"). Since it is so spongy, its surface area is very high and thus J_0 is greatly increased. Polarization effects at platinum electrodes are described in [17, 18]. Table 4.1 [3] shows values for J_0 in [A/m²].

Material	J ₀ [A / cm ²]
Au, hydrogen reaction	3.98x10 ⁻⁶
Pt, hydrogen reaction	7.94x10 ⁻⁴



 Table 4.1: Exchange current densities

4.1.8 Steady-State Diffusion Resistance

At high current densities there is a decrease of ions near the electrode since not enough ions from the bulk can diffuse to the electrode, thus building an overpotential η_d . The highest possible current density which supplies enough ions to the electrode is called the saturation current density J_s . The overpotential η_d is related to J_s by

$$J/J_{s} = 1 - e^{(-|\eta_{d}| - z/V)}$$
(4.27)

which leads to an incremental diffusion resistance of

$$R_{d} = \gamma_{d} / J = V_{t} / [z \cdot (J_{s} - J)] \qquad \text{in } \Omega \cdot m^{2} \qquad (4.28)$$

This equation is only valid for near DC conditions.

4.1.9 Warburg Impedance

Warburg proposed that the impedance due to diffusion in response to a sinusoidal forcing function varies with the frequency as

$$SZ_{a}S = \frac{k}{\sqrt{f}}$$
(4.29)

k: constant dependent on electrochemistry and mobilities of participating ionsf: frequency in Hz

This model is based on the assumption that the effect of a sinusoidal potential applied to an electrode is higher on ions close to the electrode and decreases the further away the ions are. Thus when the frequency is increased the concentration gradient of ions will increase near the interface (due to the mentioned higher forces). With increasing distance into the bulk solution the concentration gradient will decrease because of damping effects. Institut für Mikrostrukturtechnik, Universität Karlsruhe (TH)Diplomarbeit cand. mach. Udo LangMEMS Lab at the University of Washington, SeattleIntracellular Probe Device

A higher concentration permits higher current densities for a given potential. Warburg solved the diffusion equation with the presumption that the depth of the effects of the concentration wave is small compared to the thickness of the diffusion layer. His solution was a R-C series which can be transformed into a parallel R-C network. This model is illustrative since it provides a path for the DC current through the Warburg resistance. In reality such a path does not exist (see below) but it satisfies more the intuition. For an electrode which is operated near equilibrium and for a single ion species the series Warburg impedance becomes:

$$Z_{W} = R \frac{s}{W} + 1 / j \cdot \dot{\mathbf{e}} \cdot C \frac{s}{W}$$

$$(4.30)$$

where

$$R_{W}^{s} = 1/\dot{\mathbf{e}} \cdot C_{W}^{s} \tag{4.31}$$

which yields

$$SZS = \sqrt{2} \cdot R \frac{s}{W}$$
(4.32)

where

$$R_{W}^{s} = 10^{-3} \cdot \frac{V_{t}}{z^{2} \cdot q \cdot n^{0} \cdot \sqrt{4} \cdot \mathbf{\dot{a}} \cdot f \cdot D} \qquad \text{in } \Omega \cdot \text{cm}^{2}$$

$$(4.33)$$

D: diffusion coefficient (in cm^2/s)

For the parallel equivalent

$$Z_{W} = \left(1/R \frac{p}{W} + j \cdot \grave{e} \cdot C \frac{p}{W}\right)^{-1}$$

$$(4.34)$$

and as for the series above (4.23)

$$R_{W}^{p} = 1/\grave{e} \cdot C_{W}^{p} \qquad \text{in } \Omega \cdot \mathrm{cm}^{2} \qquad (4.35)$$

and

$$R_{W}^{p} = 10^{-3} \cdot \frac{V_{t}}{z^{2} \cdot q \cdot n^{0} \cdot \sqrt{\dot{\mathbf{a}} \cdot f \cdot D}} \qquad \text{in } \Omega \cdot \text{cm}^{2} \qquad (4.36)$$

For univalent ions at 25°C this equation can be written

$$R_{W}^{p} = \frac{9.044 \cdot 10^{19}}{n^{0} \cdot \sqrt{D \cdot f}}$$
(4.37)

In [19] the diffusion coefficient for ATP, sodium and potassium in cytoplasm can be found:

$$D \approx 10^{-6} \text{ cm}^2/\text{s} = 10^2 \,\mu\text{m}^2/\text{s} \tag{4.38}$$

Put into equation (4.29) this yields

$$R_W^{\ p} = 9.76 \cdot 10^{10} \cdot \frac{1}{\sqrt{f}}$$
 in $\Omega \cdot \mu m^2$ (4.39)
The limitations of the Warburg model can be seen when it is compared to the DC diffusion case (chapter 4.1.8):

$$\lim_{f \to 0} 10^{-3} \cdot \frac{V_t}{z^2 \cdot q \cdot n^0} \cdot \sqrt{\dot{a} \cdot f \cdot D} = " @R_d = ^{X_d} / ^J = V_t / z \cdot [1/(J_s - J)]$$
(4.40)

Despite this contradiction the Warburg model is the model which is commonly used by electrochemists and will thus also be used in this project.

4.1.10 Spreading Resistance

The last part that has to be derived for the theoretical model of the electrode is the resistance from the electrode into the conductive solution, the so-called spreading resistance. It is determined by the geometric area of the electrode. Generally the resistance can be derived by:

$$R_{s} = \oint_{x=0}^{x=1} dR_{s}$$
(4.41)

x: distance normal to surface

In this case there is a basically spherical source of current, namely the probe tip. Basically it is a cone but since it is small compared to the environment it will be considered to be a sphere. Thus it can be calculated by the following formula [12]:

$$R_{s} = \oint_{x=0}^{x=\cdot} \frac{\acute{\mathbf{a}}}{4 \cdot \grave{\mathbf{a}} \cdot r^{2}} dr = \frac{\acute{\mathbf{a}}}{4 \cdot \grave{\mathbf{a}} \cdot r_{s}}$$
(4.42)

r_s: radius of sphere

 $\rho{:}$ conductivity of solution, for normal saline it is 72 $\Omega{\cdot}cm$

Again a rough estimation is made here. To get an idea of r_s it is assumed that r_s is the radius of a sphere which has the surface A_M . This yields

$$r_{s} = \sqrt{\frac{A_{M}}{4 \cdot \dot{a}}} = \sqrt{\frac{55.7 \,\ddot{\mathsf{U}} \, m^{2}}{4 \cdot \dot{a}}} = 2.1 \,\ddot{\mathsf{U}} \, m \tag{4.43}$$

Put into (4.31) R_s can be calculated:

$$R_{s} = \frac{72 \, \mathbf{D} \cdot cm}{4 \cdot \mathbf{a} \cdot 2.1 \, \mathbf{U} \, m} = 27 \, k \, \mathbf{D} \tag{4.44}$$



4.1.11 Resistance of Interconnects

The interconnects are usually made of metals. The resistance of these interconnects can easily be calculated by the wellknown formula for electrical conductance:

$$R_c = \rho_c \cdot L/A$$
 (4.45)
 ρ_c : resistivity of conductor material in $\Omega \cdot m$
L: length of conductor
A: cross-sectional area of conductor

The length of the interconnects is assumed to be 3mm since that will be the probable sidelength of the chip. The width will be around 200 μ m and the height (sputtered interconnects) around 1 μ m. All these dimensions might differ from the actual probe tip but they give an idea about the order of magnitude. As material gold is presumed since it can be easily sputtered on silicon structures.

$$R_{c} = \rho_{Au} \cdot L/A = 2.24 \cdot 10^{-6} \,\Omega \cdot cm \cdot 3000 \,\mu m/200 \,\mu m^{2} = 0.34 \,\Omega$$
(4.46)

4.1.12 Parasitic Capacitances

While measuring bioelectrical events there are usually three different kinds of parasitic capacitances between the structures and the electrolyte.

Between the needle and the electrolyte:

The calculation of this parasitic capacitance is shown in [12]. The basic idea is that there is a conductive cylinder coaxially surrounded with an insulator and thus divided from the electrolyte. The formula for the calculation of this capacitance is

$$c_{s} = \frac{2 \cdot \dot{\mathbf{a}} \cdot \dot{\mathbf{e}}_{0} \cdot \dot{\mathbf{e}}_{r}}{\ln \left(\frac{R}{r} \right)} \qquad \text{in F/m} \qquad (4.47)$$

R: outside radius r: metal radius

The passivation layer is made of Si_3N_4 and covers the tip with a thickness of 1 μ m (sputtered). This technique was used in several similar applications [15, 20, 21, 22]. We have the following values for the calculation:

ε_r=7.5 [3] L=150 μm R=3.5 μm r=2.5 μm

This yields a specific capacitance of

$$c_{s} = \frac{2 \cdot \grave{a} \cdot 8.85 \cdot 10^{-14} \frac{F}{cm} \cdot 7.5}{\ln(3.5 \ddot{U} m/2.5 \ddot{U} m)} = 1.24 \cdot 10^{-11} \frac{F}{cm}$$
(4.48)

With the length of the tip L=150 μ m the actual capacitance can be calculated:

$$C_{s} = c_{s} \cdot L = 1.24 \cdot 10^{-11} \text{ F/cm} \cdot 0.015 \text{ cm} = 1.9 \cdot 10^{-13} \text{ F} = 0.19 \text{ pF}$$
(4.49)

Between electrolyte and interconnection:

In [3] it is shown that for this calculation the simple parallel-plate capacitor equation can be used if the chip onto which the probe tip will be placed is planar. Thus the capacitance is then:

$$\begin{split} C_p = &\epsilon_0 \cdot \epsilon_r \cdot L \cdot W/d & \text{ in F } \\ L: \text{ length of the interconnection} \\ W: \text{ width of the interconnection} \end{split}$$

d: thickness of insulator

The metal interconnects will be covered with a passivation layer of Si_3N_4 . The dimensions are the same as for the calculation of the resistance of the metal interconnects. Thus

$$C_{P}=8.85 \cdot 10^{-14} \text{ F/cm} \cdot 7.5 \cdot 0.3 \text{ cm} \cdot 200 \text{ } \mu\text{m}/1 \text{ } \mu\text{m}=4 \cdot 10^{-11} \text{ F}=40 \text{ } p\text{F}$$

$$(4.51)$$

Between interconnections and substrate:

In this case the interconnections are separated from the substrate by an insulation which usually is built by SiO₂ which always occurs on bare silicon surfaces. Again the equation for the parallel-plate capacitor can be used but this time with ε_r for silicon oxide (ε_r =3.9, [3]).

$$C_{s} = 8.85 \cdot 10^{-14} \text{ F/cm} \cdot 3.9 \cdot 0.3 \text{ cm} \cdot 200 \ \mu\text{m}/1 \ \mu\text{m} = 2 \cdot 10^{-11} \text{ F} = 20 \text{ pF}$$
(4.52)

4.1.13 Complete Theoretical Model

So far two parts of the complete theoretical model have been investigated. First the electrical properties of the cell and its membrane and second the processes between an electrode and an electrolyte. The latter can be derived from the previous chapters and completed to a circuit (see Fig 4.4). The behavior of the cell in an intracellular measurement can be derived as a series of the membrane properties and the resistance of the intracellular cytoplasm (cell liquid). The path from the membrane to the reference electrode defines the resistance of the extracellular liquid. Finally there is the reference electrode and the amplifier. From the amplifier to the electrode there is the resistance of the interconnects and between the electrode and the extracellular fluid there are the parasitics.

The parasitics of the part of the electrode which is intracellular has been neglected since the geometric area of the part of the electrode which is inside the cell is very small.



4.2 Electrode Noise

There is an intrinsic signal attenuation associated with the metal-electrolyte interface. This signal attenuation is called noise and has been shown to be thermal [1, 12], neural noise coupling through the deposited insulation [23] and photopotentials [23].

Thermal:

Based on Nyquist's theorem [12] the standard Johnson thermal noise equation can be used to calculate the rms (root mean square) voltage noise of a resistor:

$$V_{msnoise} = \sqrt{4 \cdot k \cdot T \cdot R_{N} \cdot \mathbf{\zeta} f}$$
(4.53)

k: Boltzmann's constant T: temperature in K

R_N: real part of electrode impedance

 Δf : bandwidth of interest





hertz since it is the bandwidth and not the actual frequency which is of interest. For practical applications the electrode impedance is measured in the bandwidth of interest and then the thermal noise can be estimated by using equation (4.53).

Neural noise coupling through insulator:

To minimize neural noise the impedance across the insulating film covering the probe tip and the interconnections must be high compared to the *electrode impedance to ground*. *This* demand *can be improved by applying an insulating lacquer over the insulation layer [13]*.

Photopotentials:

When light strikes a semiconductor like silicon carriers are generated in the space charge region and thus the surface potential will be reduced. This effect is known as photopotential. In [13] it was shown that the photopotential could be greatly reduced by the right choice of the substrate (best results with 6 Ω ·m p-type silicon).

4.3 Stimulation

In this project stimulation with an electrode is only an option and thus it will not be discussed in detail but only a few remarks and references will be given. Whereas during recording the processes at the electrode interface are mainly of capacitive nature during stimulation other reactions may occur. In this case current has to be driven through the electrolyte and thus irreversible electrochemical reactions (e.g. formation of gases) may happen which have to be avoided as much as possible. One approach is to use balanced biphasic current pulses [3]. Furthermore some examinations on maximum allowable currents through Pt-electrodes regarding the applied voltage were done by [24].

4.4 Comparison of Metal Electrodes with Glass Electrodes

Metal electrodes have a relatively high low-frequency impedance. Thus in order to measure resting potentials an amplifier with a very high input impedance is necessary (voltage drop there). In contrast the impedance of an electrolyte-filled micropipette is resistive which means that it has the same impedance over the whole frequency range. Its impedance at low frequencies is lower than that of a metal electrode but at high frequencies it is reversed. Hence metal electrodes are usually used for measuring action potentials whereas electrolyte-filled micropipettes are used for the determination of resting potentials [11].

Furthermore a metal electrode has a lower resistive part of the impedance at high frequencies and thus a lower noise voltage (see equation 4.37). For this reason high-gain recording of action potentials should be done with metal electrodes.

Summarizing all this facts the approach for this project should be an amplifier with a very high input impedance and a metal electrode with an impedance as low as possible (which might be improved by, e.g., electrodeposition of Pt "platinum black" [1]).

5 STRESS CALCULATION

In this chapter only a rough calculation of the stresses will be given since the exact final dimensions of the structure cannot be predicted at this stage of the project. These calculations will only give the order of magnitude of the forces and stresses and not the exact values. For the calculation of the stresses five different kinds of applications are investigated (see Fig. 5.1). To be able to make calculations a probable geometry of the probe will be used in the equations. In chapter 3.5 those dimensions are shown.



Since the probe will be made out of silicon, the mechanical properties shown in Table 5.1 will be used. It has to be mentioned that due to the rough estimation silicon will be treated like an isotropic material. Furthermore the material properties for silicon bulk structures and not for whiskers (small and long single crystals) will be used since the likely dimensions of the probe does not allow to use the properties of a whisker. Since all the calculations are only rough estimations the introduction of safety factors is omitted. Another reason for this is that the dimensions cannot be changed since they are determined by the goal of getting into neurons.

Young's modulus	169 000 N/mm ² [25]
Yield strength	350 N/mm ² [26]

 Table 5.1: Mechanical properties of silicon

Case 1: Pressure:

If the tip is loaded with a coaxial force the calculation has to be done for pressure force. To be on the "safe side", the following assumptions are made:

- Use of yield strength instead of compressive strength since $R_{\rm D}$ is usually higher than $R_{\rm m}$
- The stress calculation will be made for the top of the probe because there is the location of the smallest diameter of the probe.

The equation for the calculation of the stress is:

$$\mathbf{F}_{\max} = \boldsymbol{\sigma}_{\max} \cdot \mathbf{A}$$
(5.1)

 σ_{max} : maximum allowable stress (here $\sigma_{max}=R_m$)

A: area of cross section at tip, here $A=\pi \cdot r^2=\pi \cdot (d_{tip}/2)^2=3.14 \cdot (0.25 \ \mu m)^2=0.2 \ \mu m^2$

This yields

$$F_{max} = 350 \text{ N/mm}^2 \cdot 0.2 \ \mu\text{m}^2 = 0.00035 \text{ N/}\mu\text{m}^2 \cdot 0.2 \ \mu\text{m}^2 = 0.07 \text{ mN}$$
(5.2)

As already mentioned this is a worst case scenario. The actual possible load will be higher due to the assumptions made.

Case 2: Buckling while penetrating tissue:

While inserting the probe into the cell buckling might occur. It is very difficult to predict which kind of instability (Euler buckling) might damage the structure. Thus again some assumptions are made to calculate the highest possible stress.

• Buckling with one end connected to the ground and the other end freely moveable in the coaxial direction but constrained in the x-direction. This should represent the constrained flexibility in x-direction while penetrating tissue.

The corresponding formula of Euler for this kind of buckling is:

$$F_{B}=\pi^{2} \cdot E \cdot I_{y}/l_{B}^{2}$$
(5.3)

$$F_{B}: \text{ force at which buckling starts}$$
E: Young's modulus

$$I_{y}: \text{ area moment of inertia}$$
l_{B}: effective buckling length

For the calculation of the actual buckling load the following dimensions are used (see chapter 3.5):

 $h_{total} = l_B = 150 \ \mu m$ D=d=5 \ \mu m This yields

$$I_{y} = \frac{\dot{a} \cdot d^{4}}{64} \mathbb{Z} \frac{3.14 \cdot (5 \ddot{U} m)^{4}}{64} = 30.6 \ddot{U} m^{4}$$
(5.4)

Put into (5.2):

$$F_{B} = \dot{a}^{2} \cdot 0.169 \frac{N}{\ddot{U} m^{2}} \cdot 30.6 \ddot{U} m^{4} / (150 \ddot{U} m)^{2} = 2.7 mN$$
(5.5)

Case 3: Free bending:

This case might happen when lateral movements between the tissue and the probe occur at the very beginning of the insertion of the probe. The forces that then act on the probe are strictly in the horizontal direction (if only lateral relative movements are presumed). The well-known equation for the calculation of the resulting stress is:

$$\begin{split} \sigma_{max} = M/S & (5.6) \\ M: \text{ bending moment, here } F_{bend} \cdot H_{total} & \\ S: \text{ section modulus, for circular area } S = \pi \cdot d^3/32 & \\ \text{here: } S = \pi \cdot (5 \ \mu m)^3/32 = 12.27 \ \mu m^3 & \end{split}$$

This leads to

$$F_{bend} = \sigma_{max} \cdot S/H_{total}$$

$$F_{bend} = 0.00035 \text{ N/}\mu\text{m}^2 \cdot 12.27 \ \mu\text{m}^3/150 \ \mu\text{m} = 2.9 \cdot 10^{-5} \text{ N} = 0.029 \text{ mN}$$
(5.7)

Case 4: Constrained bending:

When the tip of the probe has penetrated the tissue a little bit so that it is not freely moveable any more in the lateral direction and if a relative movement in perpendicular direction to the probe between chip and tissue occurs then the case shown in Fig. 5.1 has to be calculated. In [27] the formulas can be obtained:

$$M_{A} = \frac{1}{2} \cdot F \cdot l \tag{5.8}$$

$$\hat{a}_{max} = \frac{M_{max}}{S} = \frac{M_A}{S} = \frac{\frac{1}{2} \cdot F_{max} \cdot l}{S} = \frac{F_{max} \cdot l}{2 \cdot S}$$
(5.9)

M_A: bending moment in point "A"
S: section modulus, calculated in case 3
l: length of the structure, here H_{total}
F_{max}: maximum allowable force

$$F_{max} = \frac{2 \cdot \hat{a}_{max} \cdot S}{H_{total}} = \frac{2 \cdot 0.00035 \frac{N}{U m^2} \cdot 12.27 U m^3}{1500 m} = 0.057 mN$$
(5.10)

Case 5: Shear stress:

Finally damage to the probe can happen in action. The probe will be completely inserted into tissue. Then if a relative movement perpendicular to the probe between the chip and the tissue happens the probe will be loaded with shear stress. The material property that has to be used for this calculation is the shear strength which can be derived from the yield strength with the following relationship (see Mohr's circle):

$$\tau_{max} = 0.5 \cdot \sigma_{max}, \text{ here } \tau_{max} = 0.5 \cdot 350 \text{ N/mm}^2 = 175 \text{ N/mm}^2$$

$$\tau_{max}: \text{ shear strength}$$

$$\sigma_{max}: \text{ maximum allowable stress (here } \sigma_{max} = R_m)$$
(5.11)

The formula for the calculation of the actual maximum force is:

$$\tilde{a}_{max} = \frac{F_{max}}{A} \tag{5.12}$$

F_{max}: maximum shear force
A: area of cross section, here A=
$$\pi \cdot r^2 = \pi \cdot (d_{tip}/2)^2 = 3.14 \cdot (2.5 \ \mu m)^2$$
 (5.13)
=19.63 μm^2

which yields

$$\mathbf{F}_{\max} = \tau_{\max} \cdot \mathbf{A} \tag{5.14}$$

here

$$F_{max}=0.000175 \text{ N/} \mu \text{m}^2 \cdot 19.63 \ \mu \text{m}^2=3.4 \text{ mN}$$
 (5.15)

Comparing the obtained values with values given in [5] shows that the values here are in the same range of magnitude (in [5] arrays were used). Since experiments in tissue were done in [5] without any damage to the needles, the needles in this project should be strong enough to get into neurons.

6 DESIGN

In this chapter the final design of the probe will be developed. Again the systematic approach is used which means after collecting the ideas they will be evaluated like in chapter 3. There are four problems left that have to be solved:

- Wiring (connection to data chip: kind of connection, place of connection)
- Packaging
- Conductive tip
- Fabrication process

6.1 Wiring

There are two basic problems for the connection of the probe chip to the data analysis chip. First which kind of conductor should be used and second, how should it be connected to the probe chip and third which kind of connection should be made. Again ideas were developed and then evaluated.

Conductor:

Table 6.1 shows the results found for the criteria and their weights.

Criteria	Weight
Low noise	9
Biocompatible	8
Fabrication resp. use at WTC	5
Conductive connection between chips	10
Insulated	10
Dimensions	8

Table 6.1: Criteria and weights for choosing "Wiring"

Ideas for fulfilling these goals were:

- Insulated cable
- Silicon ribbon cable [7]

In Fig. 6.1 the evaluation of these two ideas can be seen. Although alternative No. 2 was not chosen because of its complexity at the present state of the project it offers several opportunities later:

- Direct connection between probe chip and computer chip
- Direct connection between the probe chip and a far away bonding pad (see Fig. 6.2) which makes it possible to use thicker cables between the computer chip and the bonding pad

Variants	~	on noise	Soon Pa	abication of	STREET F	s la proc	Connert	
Insulated cable	+	+	+	+	+	+	Low noise if coax	50
Silicon ribbon cable	?	?	+	+	+	+	Complicated fab- rication (boron dope)	33
weight	9	8	5	10	10	8		
Weight: 1-10 resp. 1-5 for Wishes (5 resp. 10 best, 1 worst or least) Signs: ?: lack of information. !: check requirements								
Date: 03/19/99								
Böhringer, Lang			Fig	6.1	· Ch	oice	of variants for "Wiri	nơ"



Connection:

In this paragraph the design of the conductive connection between the cable and the chips is shown. The corresponding criteria and their weights are the same as for "Wiring" since the same goals have to be achieved. Possible solutions for a conductive connection are:

- Conductive glue (cable glued to surface)
- Bonding (cable bonded to surface)
- Adhesion (surface forces)
- Plug

Fig. 6.3 shows how the choice of variants was done. "Conductive glue" was chosen since it seems to be the easiest and the proven way of making a conductive connection. All variants got a "?" for insulation since it will be the same technique for all of the variants because they are all based on a bare metal to metal connection.



6.2 Packaging

Techniques that protect a chip or microsystem against its environment are called "packaging". Here the bare metal to bare metal connection mentioned in the previous paragraph has to be protected form the environment since the fluid in the brain of Tritonia diomedea is conductive. An easy solution was proposed by Russell Wyeth: the chip has to be glued with a biocompatible glue to the brain of the seaslug in order to avoid lateral movements. Since this glue is non-conductive it can also be used as an insulator which covers the conductive parts of the chip.

6.3 Bonding Pad

A bonding pad to mount the wire to the data chip has to be designed, too. There are two basic locations where the wire could be bonded:

- On the frontside of the chip
- On the backside of the chip

Since the needle is also on the frontside the wire would have to be mounted on a deeper level. Ideas for that could be a groove etched with an anisotropic wet etch or groove simply cut with a fine dicing blade as used e.g. when dicing a wafer.

For the second possibility no such a groove or trench is necessary but a conductive connection from the backside to the frontside must be made. This can be done by making a hole through the wafer, either again with a wet anisotropic etch or simply by drilling a hole through the whole thickness of the chip. In order to find the best solution experiments will have to be done (see chapter 7).

6.4 Conductive Tip

Another very important and obviously difficult problem is the removal of the passivation layer at the tip of the probe since this part has to be conductive whereas the rest of the system is covered with this passivation layer. The electrode metal will be gold and the passivation layer will be made of Si_3N_4 . The gold layer can easily be sputtered and has good covering properties [conversation with Sho Fuji] so that sharp tips will be covered with metal, too. Si_3N_4 (silicon nitride) is used since it is non-conductive but has good biocompatibility [21]. The use of gold and silicon nitride side by side is accompanied with one problem: at approximately $350^{\circ}C$ Si_3N_4 and Au have an eutecticum which might have the effect that Au is dissolved in Si_3N_4 , making the passivation layer a little bit conductive. That is the reason why LPCVD of Si_3N_4 cannot be used and instead Si_3N_4 has to be sputtered. Fabrication facilities for that are available at the microfabrication lab of the Department of Electrical Engineering.

A possibility for the removal of Si_3N_4 is the use of polyimide as a protection layer for the regions where the Si_3N_4 should still remain on the chip [personal conversation with Sho Fuji]. The exposed areas then will be etched either with a wet etch or with a dry etch (RIE). Both processes must ensure that the gold layer underneath is not attacked.



6.5 Development of Fabrication Process

At this point solutions for all of the functional units have been found. Now a suitable production process has to be found to implement these subsolutions in a microsystem. The proposed production process looks like this:

- 1. RIE in order to build probe (see Fig. 6.4)
- 2. Drilling holes near needles (for conductive connection to backside, see Fig. 6.5)
- 3. Sputtering Au frontside
- 4. Sputtering Au backside
- 5. Sputtering Si_3N_4 frontside
- 6. Spin coating polyimide
- 7. Etching Si_3N_4 (RIE or wet etch, see Fig. 6.6)
- 8. Dissolving of polyimide
- 9. Dicing of the wafer to get chips
- 10. Glueing of cable to the backside (see Fig. 6.7)









7 FABRICATION

7.1 Fabrication of Needles with RIE (Reactive Ion Etching)

7.1.1 Basics of RIE

Reactive Ion Etching (RIE) is a dry etching technique. This means that no liquid etchants are necessary. Instead of these etchants gases are used which are contained between two parallel plates. A radio frequency (RF) is applied to the bottom plate and thus accelerates stray electrons in the gas. If electrons achieve a certain velocity they are able to ionize gas atoms (thus creating electrons and positively charged ions) or to break chemical bonds (thus creating radicals). Furthermore their kinetic energy might excite atoms and then make them glow. This state in the reaction chamber is called "plasma". Depending on their properties the different components of the plasma can have either anisotropic etching effects or isotropic etching effects. The anisotropic effect takes place if the ions are accelerated towards one plate by a DC bias. They hit the wafer and thus bombard the surface (release of material). The isotropic effect is caused by reactive radicals in the gas. Since they are not accelerated towards the wafer they have the same etch rate in every direction. Fig. 7.1 shows a typical RIE reaction chamber and Fig. 7.2 shows the reactions taking place in the chamber.





The gases used in the RIE machine from Trion Technologies are SF_6 , CHF_3 and O_2 . Additional parameters for the process are RF power and chamber pressure. They can be varied with a touchscreen menu, together with the etching time. This allows to make deep structures with almost perpendicular sidewalls. Extensive experiments on this topic were mainly done at the University of Twente, The Netherlands and described in [30, 31]. Here we give a short overview of the effects of the different parameters.

<u>SF₆:</u>

 SF_6 produces the F⁻ radicals which are responsible for the isotropic etching of silicon. The chemical reactions are:

$$e^{-} + SF_{x} \rightarrow SF_{x-g} + F_{g} + e^{-} (x=3...6)$$
 (7.1)

The overall stoichiometry of etching the silicon with atomic F is:

$$Si + 4F \rightarrow SiF_4$$
 (7.2)

Obviously an increase of the SF_6 flow will lead to an increase of fluorine radicals and thus increase the isotropic, chemical etching rate.

<u>O₂:</u>

Oxygen creates O radicals reacting with the silicon and the fluorine radicals and thus building SiO_xF_y on the surface. This layer is a protection layer against the reaction of fluorine radicals with the silicon.

CHF₃:

CHF₃ is the source of CF_x^+ ions which are responsible for the removal of the SiO_xF_y layer on the bottom of the attacked wafer by forming volatile CO_xF_y . The reaction mechanism is that CF_x^+ ions compete with oxygen for free space on the silicon surface and thus hinder the formation of the SiO_xF_y layer. Since ions are accelerated perpendicularly towards the wafer they cannot hinder the formation of the protection layer on the sidewalls. This gives the possibility of an anisotropic etching profile when the sidewalls are protected with a SiO_xF_y layer against reaction with F⁺ radicals. Furthermore with increasing CHF₃ flow the oxygen in the plasma is reduced due to chemical reactions. This leads to a more isotropic etching profile [31].

Power:

Increasing the RF power increases the concentration of F radicals due to stronger collisions in the plasma as well as the ion bombardment of the surface. At low RF power the increase of the ion bombardment is larger than the isotropic etching by F thus giving a more anisotropic profile. At high RF power the increase of F becomes dominant and thus reduces the anisotropy.

Pressure:

Increasing the pressure increases the F concentration, probably due to more frequent collisions in the plasma. This increase leads to a more isotropic profile.

Etching Profiles:

In Fig. 7.3 possible etching profiles are shown. In their papers [30, 31] the group at Twente describes the profiles (from top to bottom) as negatively tapered, positively tapered and a linear profile without mask undercut. In this context they also give the definition of anisotropy which is the same for each one of the three cases:



7.1.2 Basics of Sputtering

Similarly to RIE a plasma is used. This time inert gas ions are generated and then accelerated towards the target which is at the same time the cathode. The target consists of the material to be deposited. When inert gas ions hit the surface loose target atoms are released and then condensed on the substrate. The layers obtained with this technique adhere fairly well on the substrate. Since the atoms also have a quite high kinetic energy when they reach the substrate they also have a good surface mobility [28] and thus a good step coverage. The step coverage is improved by the fact that the target is a spatially distributed source and thus the molecules maintain directionality for only roughly their mean free path (typically 1cm at a pressure of 10 mTorr) [28].



The settings used for metal deposition with the MRC Sputtersphere at the WTC are shown in Table 7.1 and 7.2. Due to many breakdowns of the machine the obtained thicknesses of the layer varied between 700 Å and 4000 Å for chromium and between 4000 Å and 6000 Å for gold.

Institut für Mikrostrukturtechnik, Universität Karlsruhe (TH)Diplomarbeit cand. mach. Udo LangMEMS Lab at the University of Washington, SeattleIntracellular Probe Device

Material	Chromium
Power	250 W
Pressure	8 mTorr
Time	15 min

Table 7.1: Settings for sputtering of chromium

Material	Gold
Power	200 W
Pressure	8 mTorr
Time	15 min

Table 7.2: Settings for sputtering of gold

7.1.3 Experiments

7.1.3.1 Experiment 1

The first experiments were done with a recipe by S. Henry et al. [5]. It shows tapered needles with a base diameter of about $35\mu m$ and a tip diameter of about $1\mu m$. The length of those probes is about 130 μm . These dimensions would fulfill all the requirements which were initially made by the project members from the Department of Zoology. Table 7.3 shows the etching conditions:

Mask material	Chromium
Power	150 W
Pressure	100 mTorr
Etch time	250 min
SF ₆ flow [sccm]	20
O ₂ flow [sccm]	15
CHF ₃ flow [sccm]	0

 Table 7.3: Settings for experiment 1

Institut für Mikrostrukturtechnik, Universität Karlsruhe (TH)Diplomarbeit cand. mach. Udo LangMEMS Lab at the University of Washington, SeattleIntracellular Probe Device

All these settings can be done on the Trion RIE at the WTC but a short break every 30 minutes has to be done [personal conversation with Sho Fuji] in order to prevent the reactor chamber from getting too warm. The next step was to make a mask for patterning the chromium. It was decided to make a mask with different structures and dimensions. Fig. 7.5 shows the result. The lines and the outer ring were added to recognize any influences of the gas flow (turbulences etc.) on the etching process. The scheme for the different structures and sizes is shown in Fig. 7.6. It was printed on transparency by the Publications Services of the University of Washington with 3600dpi.





The patterning of the chromium included the following process steps:

- 1. Nanostrip 10 minutes
- 2. Sputtering chromium with standard settings (see chapter 7.1.2)
- 3. Adhesion layer (Hexamethyldisilazane, HMD), improves the adhesion of the photoresist on the wafer
- Spin coating of photoresist (AZ 1512) with a thickness of 1.5μm (5s with 700 rpm, 30 s with 3000 rpm)
- 5. Pre bake 30min at 90°C (curing of photoresist)
- 6. Mask alignment and exposure 13.5s
- 7. Photoresist development (1 part Developer AZ 351, 4 parts water), exposure 60s
- 8. Rinse and dry
- 9. Post bake 30min at 120°C to further harden the photoresist
- 10.Cr etch approximately 15min in industrial chromium etchant
- 11.Rinse and dry
- 12.RIE oxygen etch (200s with a flow of $O_2=50$ sccm) to oxide all organic residues on the wafer, especially photoresist

After this procedure the wafer is ready for the RIE experiment with the settings mentioned above. To examine the results of the etching the microscope of the profilometer was used since it provided a three dimensional view on the structures. The following observations were thus made:

- 1. The triangle structures disappear first. The rectangle structures remain longest.
- 2. The surface of the wafer is very rough. Macroscopic as well as microscopic.

To get a better view on the results an environmental SEM (ESEM) was used. All pictures were taken under a 45° angle so that the actual height of the structure is $\sqrt{2}$ times higher than on the picture. Fig. 7.7 to Fig. 7.9 show typical structures after three hours of etching. The first picture shows that structures with a small sidelength cannot be used to build useful probes. They are attacked too heavily by the etching process. The second figure shows how some structures looked like. They had very rough sidewalls. Furthermore one can see on the picture how the Cr mask breaks away after a while. Fig. 7.9 finally shows how most of the probes look like. They were covered by a transparent layer whereas in the middle darker pillars can be seen. The conjecture was that the structures were covered by a SiO_xF_y layer [30] which led to anisotropic etching but at the same time also to rough sidewalls. The rough sidewalls in the second picture might have emerged when the development of the SiO_xF_y layer was made difficult because of gas turbulences. On every picture one can also see that the surface is covered with white flakes. This might come from the mask (small slivers) or from reaction products of the RIE etching. In any case these flakes act as small masks for the etching process and thus the rough surface might be explained since underneath these flakes no anisotropic etching reaction takes place and only isotropic etching can underetch these "micromasks".



Fig. 7.7: Circle structure with 60µm diameter after 3 hours of etching



Fig. 7.8: Circle structure with 120µm diameter after 2.5 hours of etching



Fig. 7.9: Rectangle structure with a sidelength of 120µm after 3 hours of etching

In order to determine whether the transparent material is really SiO_xF_y an etch with an additional CHF₃ flow of 15 sccm for 15 min was done. Fig. 7.10 shows as result that this material was etched away as described in [30, 31] and thus the transparent material was SiO_xF_y and the dark structure was bulk silicon. This made clear that the recipe was not suitable for getting sharp, high aspect ratio structures with smooth sidewalls.



additional CHF3 flow of 15sccm

7.1.3.2 Experiment 2

In order to achieve the new requirements for the etching process a recipe proposed by the MEMS group in Twente, The Netherlands, was used [31]. In their paper they describe the results of this recipe as follows:

- Smooth surface
- Anisotropic etching profile
- No outward sloped etching profile (like in Fig. 7.3 B))

The recipe itself is shown in Table 7.2. As the mask material again chromium was used although it was not used in [31] but in the same article it was mentioned that the etching result should not be dependent on the mask material (SiO_2 , Cr).

Mask material	Chromium
Power	140 W
Pressure	60 mTorr
Etch time	60 min
SF ₆ flow	40 sccm
O ₂ flow	6 sccm
CHF ₃ flow	17 sccm

Table 7.4: Settings for experiment 2

During the etching it was not possible to get a stable plasma with these settings but it was fading in and out because of probably too low gas flows. Furthermore the chromium mask disappeared after 60 min (probably due to the high CHF_3 flow) so that deeper structures were not possible. Fig. 7.11 shows a typical structure after 60 minutes of etching. Contrary to the expectations the sidewalls are still fairly rough. The positive result of this experiment is that slight underetching at the top of the structure can be seen.


So the following conclusions can be drawn:

- Recipe 1 has the advantage that the chromium mask remains very long on the wafer (high selectivity)
- Recipe 2 has the advantage that due to the \mbox{CHF}_3 flow the $\mbox{SiO}_x\mbox{F}_y$ layer is removed
- There is slight underetching with recipe 2. This is necessary to get sharp structures

The consequences of these facts are:

- Increase of the underetching
- Add CHF₃ to get smoother sidewalls, but not too much to avoid disappearing of the chromium mask

7.1.3.3 Experiment 3

The next experiment should help to achieve the goals mentioned in the previous chapter. To do so recipe 1 was chosen again because of its long etch times without loosing the chromium mask, but with the following changes:

- Decreasing of the pressure. This will help to get more underetching [30, 31]
- Lower CHF_3 flow than in experiment 2 to avoid the disappearing of the chromium mask

The experiment was thus done with the following settings:

Mask material	Chromium
Power	150 W
Pressure	100 mTorr
Etch time	90 min
SF ₆ flow	20 sccm
O ₂ flow	15 sccm
CHF ₃ flow	2 sccm

 Table 7.5: Settings for experiment 3

In contrast to the previous experiments this time the surface seemed to be very smooth, macroscopically as well as microscopically. Once again the chromium disappeared rather quickly, in this experiment after 90 minutes. Fig. 7.12 and 7.13 show that the sidewalls are smoother now than before and that there was more underetching under the chromium mask since the structures are more tapered now. Remarkable is that there are again white flakes on the surface (probably slivers from the chromium mask). For the next experiments the remaining time of the chromium on the wafer has to be increased in order to get sharper (longer underetching) and taller structures.





7.1.3.4 Experiment 4

In order to keep the chromium longer on the wafer the CHF₃ flow was reduced. The rest of the settings are the same as in experiment 3. During this experiment it took 70 minutes until the chromium was gone. Fig. 7.14 shows a typical structure which was obtained at the end of the etching. Again the sidewalls are very smooth and a white fluff covers the surface. But again the underetching is not enough as well as the etching is not long enough to get high structures.

Mask material	Chromium
Power	150 W
Pressure	100 mTorr
Etch time	70 min
SF ₆ flow	20 sccm
O ₂ flow	15 sccm
CHF ₃	1 sccm

Table 7.6: Settings for experiment 4



Fig. 7.14: Circle structure with a diameter of 80µm

7.1.3.5 Experiment 5

In order to get a more isotropic and thus tapered profile the SF_6 flow has to be increased. Furthermore, to get smooth sidewalls the previous CHF_3 flow of 2 sccm is used again and the O_2 flow reduced.

Mask material	Chromium
Power	150 W
Pressure	100 mTorr
Etch time	90 min
SF ₆ flow	25 sccm
O ₂ flow	10 sccm
CHF ₃ flow	2 sccm

Table 7.7: Settings for experiment 5

This time a quite good result was achieved. The sidewalls are fairly smooth and the structures are at the same time high and sharp with tip diameters of about 3μ m (Fig. 7.17). Again there is a lot of white fluff on the surface of the wafer which might come from the chromium mask. Fig. 7.15 and Fig 7.16 show how the chromium mask is underetched. This time the chromium mask was etched away after 90 minutes which is an improvement compared to previous experiments but in order to get higher structures it should still be increased.



 $40 \mu m$ after an etch time of 1 hour



Fig. 7.16: Circle structure with a diameter of 80µm after an etch time of 1 hour



7.1.3.6 Experiment 6

In this experiment it was investigated whether a mixture of two recipes might give good results. A cycle consists of an etch of 10 minutes with a recipe similar to that of experiment 1 and an additional CHF₃ flow for 5 minutes (for removal of the SiO_xF_y layer).

Mask material	Chromium
Power	150 W
Pressure	100 mTorr
Etch time	60 min
SF ₆ flow	25 sccm
O ₂ flow	10 sccm
CHF ₃ flow	0 sccm for 10 min/ 3 sccm for 5 min

 Table 7.8: Settings for experiment 6

Again the chromium was etched away before sharp structures could develop. Probably the CHF_3 flow was too high although it only lasted for 20 minutes all together. Fig. 7.18 shows a typical structure obtained with this recipe. The sidewalls are very rough again, probably due to the etch with the recipe similar to that of experiment 1. Thus the recipe used in experiment 5 should be used for future improvements.



7.1.3.7 Experiment 7

To investigate the influence of the mask material on the results for this experiment a gold mask was used (thickness of the layer as described in chapter 7.1.2 5 kÅ). Furthermore the recipe of the so far best experiment (5) was used.

Mask material	Gold
Power	150 W
Pressure	100 mTorr
Etch time	55 min
SF ₆ flow	25 sccm
O ₂ flow	10 sccm
CHF ₃ flow	2 sccm

 Table 7.9: Settings for experiment 7

This time the result was completely different from the previous experiments. The wafer surface turned black which means that so called black silicon had been built up. It appears black because small particles (SiO₂ or in this case probably gold from the mask) fall on the ground and act as masks themselves. After a while the surface is covered with numberless small needles, the so called grass. It is created when the oxygen flow is high enough to protect these needles with a SiO_xF_y layer from being etched away and an anisotropic etch profile thus develops. When the length of these needles exceed the wavelength of the incoming light then the light is "caught" in the areas between the needles and the surface appears black [30]. Fig. 7.19 shows a result of the experiment. The picture was taken after an etch time of 45 minutes. It shows how rough the surface and the sidewalls are and the gold layer which was well underetched. After an additional 10 minutes etch all the gold was etched away without providing sharp structures. This approach also seems promising if the gold layer remained longer on the wafer and if the rough surface might get smoother. A possibility to achieve this might be an etch with BOE (buffered oxygen etch, diluted HF).



7.1.3.8 Experiment 8

A completely different approach was proposed by Sho Fuji, the lab manager of the Washington Technology Center (WTC). Some experiments to get deep trenches with an isotropic profile were done by the WTC in 1996. In that experiment photoresist was used as a mask for the RIE. This implies that oxygen could not be used as an etching gas but only SF_6 and CHF_3 . Since the profile should be isotropic only SF_6 was used. The first test with photoresist was made with AZ 1512 like for the mask patterning. This photoresist has a thickness after spin coating of about 1.5 μ m.

Mask material	Photoresist AZ 1512 1.5µm
Power	100 W
Pressure	200 mTorr
Etch time	60 min
SF ₆ flow	50 sccm
O ₂ flow	0 sccm
CHF ₃ flow	0 sccm

Table 7.10: Settings for experiment 8

After 60 minutes the photoresist was completely gone. The structures were not very deep but the surface and the sidewalls were very smooth. In Fig. 7.21 one can also see an interesting detail. It might give the explanation why the sidewalls of many circle structures are rougher than those of rectangle structures: the circle is not exact but due to the print program or printer a polygon with several edges. Later the RIE etching process took place along those edges. One can also see how the mask is underetched. This approach can be continued if the mask can be made thicker. The photoresist AZ 4620 which is more viscous than the AZ 1512 permits layers with a thickness of up to 12μ m and will thus be used in later experiments.



7.1.3.9 Experiment 9

Since the best results have been obtained with the recipe of experiment 5, this recipe is the basis for this experiment. The only change is that the SF_6 flow is higher than before (30 sccm instead of 25 sccm) and the O₂ flow is lower (5 sccm instead of 10 sccm) to get a more isotropic etching profile.

Mask material	Chromium
Power	150 W
Pressure	100 mTorr
Etch time	80 minutes
SF ₆ flow	30 sccm
O ₂ flow	5 sccm
CHF ₃ flow	2 sccm

 Table 7.11: Settings for experiment 9



Fig. 7.22: Rectangle structure with a sidelength of 80µm after 80 minutes of etching





As can be seen in Fig. 7.22 right after the etching process the surface was covered again with white fluff. It can also be seen that the chromium mask was fairly underetched and the white flakes accumulate underneath the mask. In Fig. 7.23 a good structure after rinse and dry can be seen. The tip is quite sharp but not optimal yet. The sidewalls near the tips are quite smooth whereas near the ground they are rougher. Since this did not happen with a gold mask it might have something to do with the chromium. Fig. 7.24 shows that the circle structures are usually too much underetched so that a sharp and high probe is not feasible. The results, especially those of the rectangular structures, are very promising so that this recipe will be used for further improvement. But this also shows again that it is essential that the chromium remains on the wafer as long as possible.

7.1.3.10 Experiment 10

As mentioned in chapter 7.1.8 a thicker photoresist (AZ 4620) was used this time. It could be spun up to 12μ m thick. But in this experiment a thickness of 6μ m was used (spinning 5s at 500rpm and 40 s at 5000rpm) together with settings from the recipe of experiment 8.

Mask material	Photoresist AZ 4620, 6 µm thick
Power	100 W
Pressure	200 mTorr
Etch time	160 min
SF ₆ flow	55 sccm
O ₂ flow	0 sccm
CHF ₃ flow	0 sccm

 Table 7.12: Settings for experiment 8

Since the plasma was not stable with a SF_6 flow of 50 sccm this flow was increased to 55 sccm and the RIE mode of the machine was set to manual.





Fig. 7.26: Rectangle structure with a sidelength of 80µm after an etch time of 160 minutes



etch time of 160 minutes

Fig. 7.25 to Fig 7.27 show the different states of the process. The pillar is mainly underetched in the middle until it finally becomes a sharp needle with a tip diameter of less than a micron. If it is too thin or etched too long it might break as can be seen in the last picture.



Finally the last two pictures show the results with the circle structures. Again the structure is underetched mostly in the middle of the pillar. If it is underetched too long, it might break as can be seen in Fig. 7.27. Furthermore as mentioned in chapter 7.1.7 the edges due to the polygonal nature of the circles can be seen. The white flakes that can be seen on all of the pictures are the remainders of the photoresist.



7.1.3.11 Experiment 11

After the RIE spin coating is necessary (for the polyimide). That is the reason why the original mask cannot be used any longer and a second mask has to be designed. As described in chapter 7.1.3.3 the following facts have become evident in each experiment so far:

- The patterns need a sidelength or diameter of at least 60µm to result in structures that are high enough
- The best results were obtained with rectangular structures

Furthermore the other processes have to be considered as well. This includes especially the drilling of the holes and the spin coating of the polyimide. Thus enough space between each probe has to be included and the line structures have to be omitted because they might influence the spin coating.

Taking into consideration all these constraints it was decided to use rectangular structures with a sidelength of at least 80µm and a gap of 5.5mm between each probe in each direction. The new layout can be seen in Fig. 7.30. It is an asymmetrical array in order to know later on which side the bigger structures are. Fig. 7.31 shows the scheme of the mask which is designed for 3" wafers.





The first experiment was then done with the settings and the etching time of experiment 13 to see whether the results with the new mask would be the same as with the old one.

Mask material	Photoresist AZ 4620, 6µm
Power	100 W
Pressure	200 mTorr
Etch time	160 min
SF ₆ flow	55 sccm
O ₂ flow	0 sccm
CHF ₃ flow	0 sccm

 Table 7.13: Settings for experiment 11

Again, during the experiments the machine had to be set to manual mode since the plasma was fading in and out. As can be seen in Fig. 7.32 the etching conditions with new mask have changed. The photoresist was etched too soon so that no sharp structures were achieved. Thus in the next experiment a thicker mask was used.



7.1.3.12 Experiment 12

In this experiment a thicker mask (AZ 4620 with a thickness of 12μ m) was used in order to etch longer. This was necessary since in the previous experiment the photoresist disappeared too soon before any sharp structures were obtained. Thus now an etching time of 180 minutes was chosen.

Mask material	Photoresist AZ 4620, 12µm
Power	100 W
Pressure	200 mTorr
Etch time	180 min
SF ₆ flow	55 sccm
O ₂ flow	0 sccm
CHF ₃ flow	0 sccm

Table 7.14: Settings for experiment 12



Again the machine was set to manual mode because of the plasma fading in and out. This time good structures with originally 80µm sidelength were obtained all over the wafer so that now a good production process for the needles was found.



7.2 Drilling of Holes

Since there has to be a conductive connection from the frontside to the backside holes have to be drilled into the wafer. Since silicon is a brittle but hard material only drilling bits with diamond tips can be used. Furthermore they have to be cooled with water during the actual drilling process. It was soon realized that the wafers are too brittle for drilling holes directly and thus break. In order to avoid this glass slides are glued with crystal bond on both sides of the wafer and then the holes are drilled. After that the crystal bond can be dissolved in acetone. Since the bonding of glass slides on the needles is not possible this step has to be done first, even before the nanostrip. In order to know where to drill the mask was printed on paper and then attached on the wafer and thus used as a drilling mask.

7.3 Sputtering of Gold

The next process step is the sputtering of the electrode material, in this case gold, onto the wafer. The settings for the MRC Sputtersphere to obtain a 5000 Å thick layer are shown in table 7.2.

Then the adhesion of the gold layer to the silicon substrate was tested with the "Scotch test", the sticking of an adhesive tape on the surface. If the layer is removed with the tape then the adhesion is poor. This was the case here. It could also be removed easily with a sharp tool. Thus as described in [15] a chromium layer was sputtered first and then the tests were repeated. The settings for a approximately 2000 Å thick layer are shown in table 7.1.

This time the adhesion was much better so that it will be used from now on as a basis for the gold layer. Furthermore as can be seen in Fig. 7.35 the tip was probably not covered with gold. In Fig. 7.36 there is only chromium sputtered on the substrate. The tip seems to be covered with chromium. The result of sputtering gold on chromium can be seen in Fig. 7.37. Again the gold probably does not cover the whole tip (small pearls or drops instead of a layer). In latter applications the goal should be that the tip is driven far enough into the cell so that the continuous gold layer can record intracellularly.

In addition a test was made whether a conductive connection between the backside and the frontside can be obtained through the drilled hole. Holes were drilled through the wafer with drill bits with

diamond bits and then a chromium and a gold layer were sputtered on the frontside and the backside. The layers around the holes on one side were then disrupted with a diamond cutter and then the conductivity was measured between the area near the hole and the other side with a multimeter. Since it was conductive (resistance almost zero) it proves that an interconnection through the hole existed. Thus the overall production process can be done as planned.







7.4 Sputtering of Si₃N₄

A layer of Si_3N_4 was used for passivation since it has good biocompatibility [1]. It was sputtered on the wafer to avoid an eutecticum with gold at 350 °C. The sputtering process takes place at room temperature. For this process the Perkin-Elmer sputtering machine at the Microfabrication Lab of the Electrical Engineering Department had to be used with the settings in table 7.15.

Base pressure	7.10 ⁻⁶ Torr
Forward power	125 W
Gas mixture	Ar 20 mTorr, N ₂ 6 mTorr
Target bias	1000 V
Deposition pressure	8 mTorr
Deposition rate	100 Å/min

Table 7.15: Settings for Si₃N₄ sputtering



A short first test run with old structures was then done with a deposition time of 35 minutes. The results are shown in Fig. 7.38. The surfaces were completely covered with silicon nitride. But the picture also shows that the needles have to be handled with care since here it was broken apart. Furthermore a conductance test with a multimeter was done which showed that the layer isolates the layers underneath it and thus achieves its goal.

7.5 Spinning of Polyimide

The spinning of the polyimide (PI 12721) to get an approximately 40μ m thick layer the following processes were necessary. They are standardized by the manufacturer of the resist.

- 1. Adhesion layer (described in chapter 7.1.3.1)
- 2. Pouring of the polyimide on the wafer until the whole surface is covered
- 3. Kick off at 10,000rpm for 3s
- 4. 30s at 1000 rpm
- 5. Evaporation of solvent: 3 min at 65°C and 3 min at 90°C
- 6. Flood exposure under the ABM aligner 22s (since the PI 12721 is negative resist all the exposed areas in this case the whole wafer will remain on the wafer)
- 7. Developing: 50s in DE 6180
- 8. Rinse: 20s in DE 9180

Fig. 7.39 shows how everything but the needles was covered with the polyimide. To avoid that a very thin polyimide layer covered the needles during the removal of the Si_3N_4 a short oxygen etch was done before that. Fig. 7.40 shows a needle after a 20s oxygen etch. The polyimide can be removed by an RIE oxygen etch or with Nanostrip. If Nanostrip is used structures and layers can be damaged. Thus RIE was used.





7.6 Removal of Si₃N₄ from the Actual Prototype

The removal of the Si_3N_4 was done with RIE. A basic recipe for this process is described in [32] and is shown in table 7.16. It could be used in this application since the O_2 flow was zero and thus no organic material such as polyimide was attacked. Furthermore it had to be assured that the gold layer was not attacked by this process. Fig. 7.41 shows the tip after a 12 minute etch under these conditions. No apparent changes can be seen and thus the process could be used for the removal of the silicon nitride. Since the mask for the Si_3N_4 etch was made with polyimide it also had to be investigated whether it was attacked under these conditions. Again no changes could be observed. This means that we can be confident that this technique can be used for the project.

Power	100 W
Pressure	250 mTorr
Etching rate	200 nm/min
SF ₆ flow	50 sccm
O ₂ flow	0 sccm
CHF ₃ flow	0 sccm

Table 7.16: Settings for removal of Si_3N_4



7.7 Dicing

The complete wafer was then diced into chips with a sidelength of approximately 5mm by 5 mm. Hereby the gold layer was damaged and torn apart along the dicing line whereas the chromium layer remained on the wafer.

7.8 Wiring

Finally simply an insulated wire for the connection to the analysis chip had to be connected with conductive glue to the backside of the probe chip. It was then covered with epoxy glue for protection and insulation.

8 EXPERIMENTS

The first experiments with the prototype were done at Friday Harbor Laboratories on the San Juan Islands. Due to a breakdown of the silicon nitride sputtering machine a thin layer of the photoresist AZ 1512 was spun on the wafer as a passivation layer. Fig 8.1 shows a prepared chip. A cable was glued to the backside as described in chapter 7.8 and then covered with a plastic tube. At the end a connector was soldered to the cable. The following experiments were performed:

Measurement of the resistance:

The prototype was fixed with a manipulator and a very fine syringe filled with saltwater (almost the same properties as saline solution) was brought with another manipulator over the needle. Then the needle was immersed in the solution and the resistance was measured. This was done with a 1nA current and the voltage drop along the electrode was measured. In chapter 4.1.6 a charge transfer resistance (largest part of overall resistance) of $R_t=1.2\cdot10^{10} \Omega$ was calculated. Since the photoresist was spun on the surface and did not cover the sidewalls of the needles the electrode area was much bigger than in the assumptions for the calculations in chapter 4. Thus R_t should be smaller in this experiment than in the calculations. The measured value was $5\cdot10^9 \Omega$ and thus shows the correct order of magnitude of the calculations. It is still too high for practical application in intracellular recording experiments but with an electrodeposition of platinum black as previously mentioned (chapter 4.1.7) this resistance can be lowered by several orders of magnitude.

Intracellular potential:

The tests were done with barnacle muscle cells. With the first prototypes no clear data could be obtained. It turned out that the originally demanded length (by Prof. Willows and Russell Wyeth) of the needles of 100μ m was not enough. It was then proposed by Prof. Willows and Russell Wyeth to have a length of at least 300μ m for the needles to be sure that the needle is inside the cell. The chip was driven directly and perpendicularly into the tissue. After the experiment the needle was examined under a light microscope and no damage to the needle could be seen. This means that the mechanical strength of the needles is high enough as predicted by the calculation in chapter 5.

Furthermore the next experiments have to be done with a better passivation layer (Si_3N_4) since the photoresist layer does not insulate well enough (ions from the fluid can leak through the photoresist to the metal layer).



9 SUMMARY AND PERSPECTIVES

In this thesis a new approach towards neural recording was shown. Instead of measuring in the lab and extracellularly a prototype was developed. This probe chip can be implanted into the brains of live, freely behaving sea slugs of the species "Tritonia diomedea". The probe chip is connected to an analysis chip that compresses and records the obtained data.

The first step towards a probe chip was to make a list of goals which had to or which should be achieved. After systematically finding possible solutions the theoretical basics of electrodes and their mechanical strength were developed. Then the detailed fabrication process was developed and tested. The probe chip itself finally consisted of a silicon substrate and an electrode which is shaped like a needle made by a RIE process. It was then covered with a chromium layer to improve the adhesion of the top gold layer. This layer is the actual electrode. It was then covered with a passivation layer of silicon nitride which was etched away again at the tip of the needle by using polyimide as a protection layer for the rest of the wafer. The first experiments in animals were done at the Friday Harbor Laboratories of the Department of Zoology, University of Washington. Until now the experiments have shown that the following tasks should be done during the upcoming stages of the project:

Connection to data chip:

Gluing the cable to the chip is macroscopic and manual work. It would be better if this could be implemented into a fabrication step within the overall process. In [7] such a possibility was shown. Boron doping was used to pattern silicon ribbon cables. Such a process would also allow to make smaller chips, only restricted by the accuracy of the dicing saw for the final separation of the chips (also see chapter 6.1).

Conductive connection from the frontside to the backside:

Another macroscopic manual work is the drilling of the holes. Since very small holes are essential this step should also be integrated in a micromechanic fabrication process. Possibilities for this are a deep RIE process or an anisotropic wet etch of a (110) wafer to get perpendicular sidewalls.

Avoidance of conductive layers at the sidewalls of the chip:

So far there have been very thin conductive layers in connection to the fluid surrounding the chip because the gold layer was sputtered on the whole wafer before dicing. In order to avoid this a metal lift-off could be done. Before sputtering the metals on the wafer a photoresist layer is spun on and then patterned in a way that it remains only on both sides along the later dicing lines. These areas will be dissolved after sputtering and thus the gold layer and the chromium layer are removed from these areas.

Size and handling of the chip:

So far the chip has been made with a size of 5mm x 5mm and a thickness of 1mm. This chip is too big since it already covers the entire brain of the sea slug. Thus it has to be reduced to a size of about 1mm x 1mm and a thickness of around 500µm. Dicing the wafer into chips of this size will not be a problem with a thin dicing blade but the handling will be more difficult.

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