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H Witte, C Warnke, T Voigt, A de Lima, I Ivanov, et al.. AlGaN/GaN-based HEMTs for electrical stimulation of neuronal cell cultures. *Journal of Physics D: Applied Physics*, 2011, 44 (35), pp.355501. 10.1088/0022-3727/44/35/355501 . hal-00649059

**HAL Id: hal-00649059**

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Submitted on 7 Dec 2011

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# AlGaN/GaN-based HEMTs for electrical stimulation of neuronal cell cultures

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PACS: 73.40.Mr, 73.61.Ey, 85.30.Tv, 87.85.eg, 87.85.dh, 87.18.Sn

Keywords: AlGaN/GaN field effect transistor, biosensor, electrode stimulation, neuron networks

Unipolar source drain voltage pulses of GaN/AlGaN - High Electron Mobility Transistors (HEMT) were used for stimulation of cultured neuronal networks obtained from embryonic rat cerebral cortex. The HEMT sensor was grown by metal organic vapour phase epitaxy on 2 inch sapphire substrate consisting of 10 single HEMTs concentrically arranged around the wafer center. Electrolytic reactions between the HEMT sensor surface and the culture medium were not detected by using cyclic voltammetry.

During voltage pulses and resulting neuronal excitation, capacitances were recharged giving indications of the contributions of the AlGaN and the AlO<sub>x</sub> isolation layers between the two dimensional electron gas (2DEG) channel and the neuron culture. The resulting threshold current for stimulation of neuron activity strongly depends on the culture and HEMT position on the sensor surface under consideration which is caused by different impedances of each neuron culture and position within the culture. The differences of culture impedances can be explained by variations of composition, thickness, and conductivity of the culture areas.

## 1. Introduction

Neuronal cell cultures are a good model to investigate the development of neuronal networks under experimentally defined conditions /1/. The stimulation and recording of neuron activity using planar microelectrodes is very helpful for studying the signal transfer within and into such network /2/. Electrode interfaces mostly used are the metal-multi electrode arrays (MEAs) on glass or silicon substrates /3/, /4/ and various types of Field-Effect-Transistors (FETs) on Silicon substrates /5/. Specially, MEA systems allow the multi site stimulation and recording from neuronal networks /6/.

Another approach for a neuron-transistor-interface represents the electrolyte gated high electron mobility transistor (HEMT) based on the AlGa<sub>N</sub>/Ga<sub>N</sub> heterostructure /7/. Polarisation charges within the AlGa<sub>N</sub>/Ga<sub>N</sub> interface create a two dimensional electron gas which acts as a conductive path directed parallel to the surface. Surface charges at the opened gate area control the conductivity of the two-dimensional electron gas (2DEG) measurable as a change in the source-drain-current /8/.

The group-III nitride materials exhibit some advantages as biosensors in neuronal networks. The excellent biocompatibility and the high chemical resistance allow the operation under biological and chemically hazardous conditions in a long time regime. Furthermore, by using HEMT structures the current can be gained by biasing the source-drain channel. These advantages are combined with the chemical sensitivity of AlGa<sub>N</sub>/Ga<sub>N</sub> devices. For instance, with HEMT structures pH-values and the conductance of electrolytic solutions /7/ can be detected /9/, as well as gases like oxygen and carbon dioxide by using functionalized surfaces /10/ or specific contents in biological cultures, such as proteins, enzymes, and cells by using marker molecules /10/. Until now there are not many reports about the use of HEMT structures as interface for the direct stimulation of neuronal networks. In /11/ the recording of cardiac myocyte cells of embryonic Wistar rats have been described and /12/ reports about the identification of extracellular potentials of NG108-15 nerve cells by an AlGa<sub>N</sub>/Ga<sub>N</sub> HEMT. In both cases Ag/AgCl reference electrode was used within the neuron culture. But, an additional reference electrode complicated the handling of the HEMT sensor during cultivation and measurements. In our study neuronal networks were cultivated on an AlGa<sub>N</sub>/Ga<sub>N</sub>- (HEMT) arrangement and were stimulated by electrical pulses delivered through the HEMT. Only the source and drain contacts of the HEMT structures were used for current stimulation.

## 2. Experimental Details

The HEMT structures are grown by metal organic vapour phase epitaxy on double polished 2 inch sapphire substrates with the layer arrangement sapphire substrate / Ga<sub>N</sub> buffer layer/ undoped Ga<sub>N</sub>/AlGa<sub>N</sub>/Ga<sub>N</sub> top layer with an Al-content of 27 % in the AlGa<sub>N</sub> layer. The 2DEG shows a sheet electron concentration of about  $2 \cdot 10^{13} \text{ cm}^{-2}$  and a mobility of about  $850 \text{ cm}^2/\text{Vs}$ . The source drain electrodes distance is 20  $\mu\text{m}$ . The contacts consist of Au/Ti layers connecting the 2 DEG channel directly. The contact feed lengths are 20 mm and the contact pad areas are  $(0.8 \cdot 0.9) \text{ mm}^2$ . All contacts

and the contact feeds are isolated by a thin 35 nm AlO<sub>x</sub> layer (resistances in the range of GΩ), whereas the gate contact area (see G in figure 2A) is free from the isolation layer and thus in direct contact with the cultivated nerve cells and the surrounding culture medium. All HEMTs show very similar IV-characteristics with a saturation current at about 35 mA at 10V and a slope below 2 V between 5.5 mA/V and 5.8 mA/V.

The wafer sensor (prepared by MicroGan GmbH) contains 10 HEMT structures arranged around the wafer centre with a distance of 10 mm between opposite HEMTs. For cell culturing the wafer is glued into a petri dish and the petri dish is placed into a custom made holder containing solder posts (see figure 1). The source-drain contact pads are connected to special base plates onto the sensor area by gold wires and further towards the solder posts by copper wires.

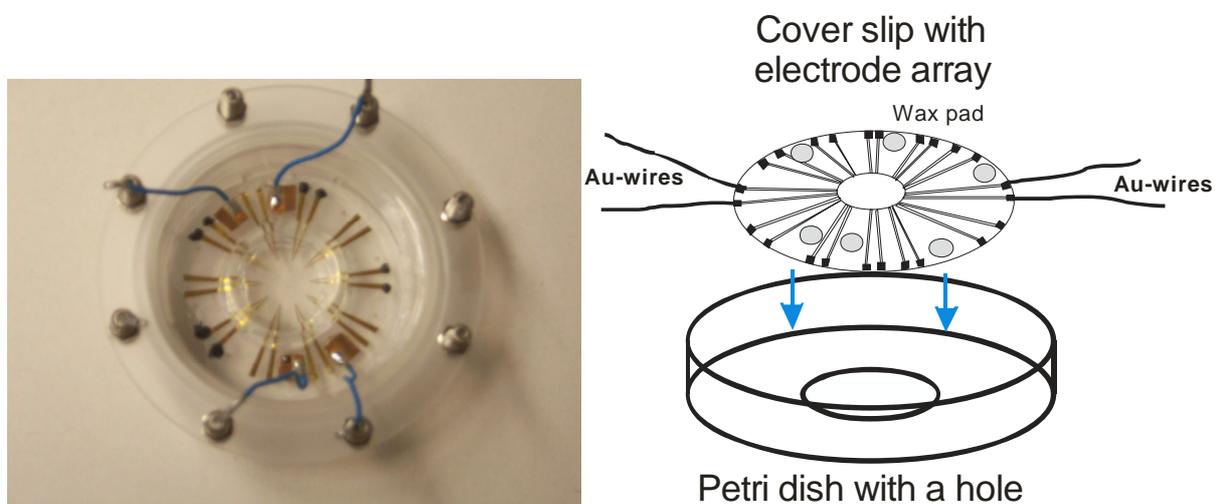


Figure 1: Picture (left) and scheme (right) of the wafer sensor contains 10 HEMT structures on a two inch sapphire wafer. The wafer sensor is glued in a petri disc and into a holder involving the solder posts.

Our HEMT structures show a high sensitivity to the pH-value for acid solutions (1.9 Ohm/pH). In contrast, at frequencies higher than 1 kHz the impedance is independent of pH-values but correlates well with the conductance of the sodium chloride and hydrochloride acid solutions as shown in (see details in /9/).

We used cyclic voltammetry to test whether the AlO<sub>x</sub> insulating layer completely prevents contact between the culture and the gold layer and to investigate the surface reactions between the open gate area of the HEMT which includes also two small stripes of bare gold contacts (see areas C in figure 2A). All measurements were made in a Pyrex electrochemical cell using potentiostat PGSTAT302 (Eco Chemie/Autolab, Netherlands); the interconnected source and drain contacts were used as the working electrode. A platinum wire represents the counter electrode and a saturated calomel electrode (SCE) the reference electrode. All potentials are applied versus the SCE.

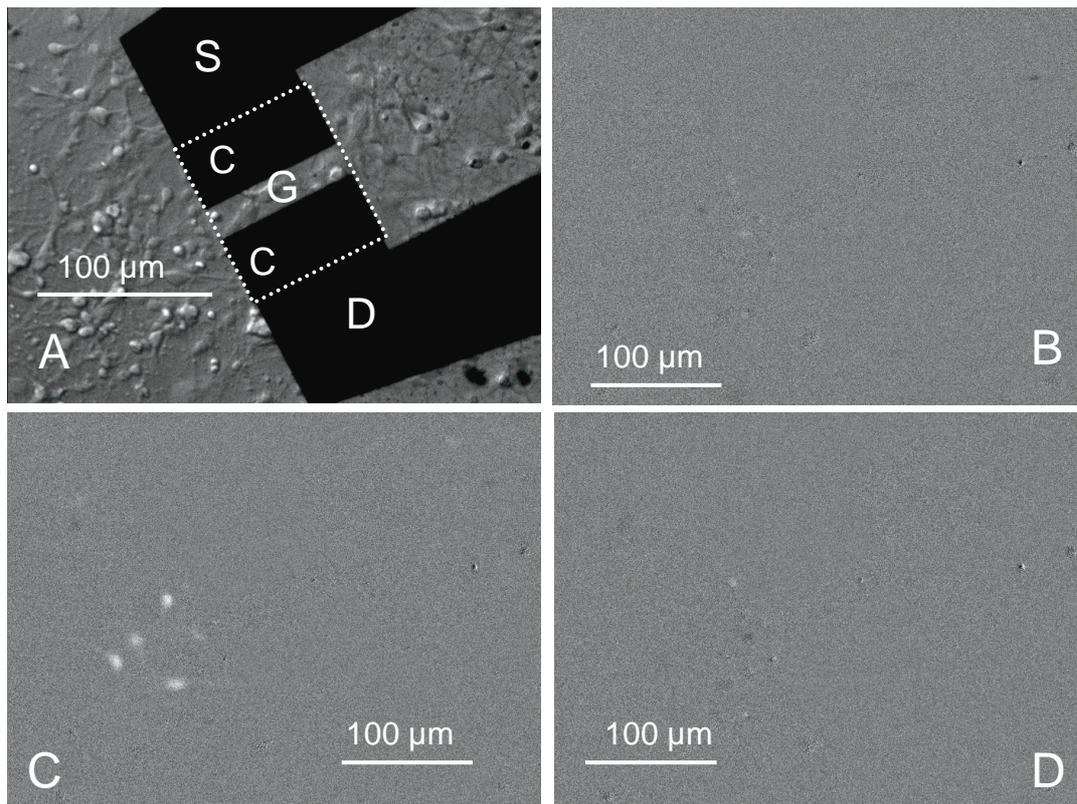


Figure 2: (A) Neurons grown on a HEMT structure. The source (S) and drain (D) contacts as well as the contacts feeds can be seen. The white frame represents the open gate area (G) and the bare gold regions (C). (B – D) Calcium imaging of spontaneous network activity of the field shown in (A): 2s before (B) during (C) and 2s after (D) a synchronous depolarization in five neurons. The spontaneous depolarization of the neurons shows that they are viable and electric active.

For impedance measurements a precision LCR meter (HP 4284) in the frequency range between 20 Hz and 1 MHz at zero bias and with an AC measurement voltage of 30 mV is used. The source- drain current is measured with a source measure unit (Keithley 236) at a bias voltage of 0.1 V. For unipolar pulse experiments the voltage of the Source Measure Unit is switched from +0.1V up to +2V and back to +0.1 V with a pulse width of 100 ms and a pulse rising time of 1 ms.

To grow nerve cells directly on the HEMT sensors, a cultivation wall is formed by gluing a 20mm acrylic glass ring over the central sensor area. To improve cell adhesion and long term neuronal survival the cultivation area is coated with Poly-D-Lysine and purified astrocytes are plated inside the acrylic glass ring one week prior to the neuronal cells. After the glial cells are grown to confluence, neurons dissociated from embryonic rat cerebral cortex are plated on top of the glia feeder layer in a density of 800 cells/mm<sup>2</sup>. Neuronal cells are cultivated for up to 3 weeks in DMEM medium in a gassed incubator with 5% carbon dioxide content, humidity of 100% and a temperature of 37 °C.

During the cultivation time neurons formed richly interconnected, electrically active networks. The spontaneous neuronal activity and their response to electric stimulation were accessed by optically recording the changes in the intracellular calcium concentration with the calcium sensitive dye Fluo-3AM./1/.

### 3. Results and Discussion

To test for biocompatibility neurons were plated and cultivated on the sensor surface and the development of spontaneous network activity was recorded by calcium imaging (figure 2). Neurons grew uniformly on the entire wafer surface including the contact zones, the region between the source drain and on the contact feeds (see areas C in figure 2A). We have qualitatively inspected the cell growth of all HEMTs on the wafer sensor and found no apparent preferences in the cellular distribution. Neurons showed spontaneous changes in their intracellular calcium concentration (figure 2B-D), corresponding to the firing of action potential bursts /1/

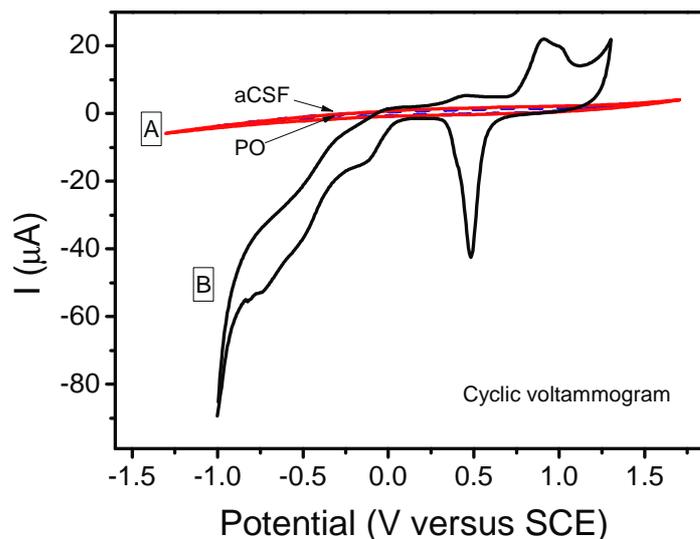
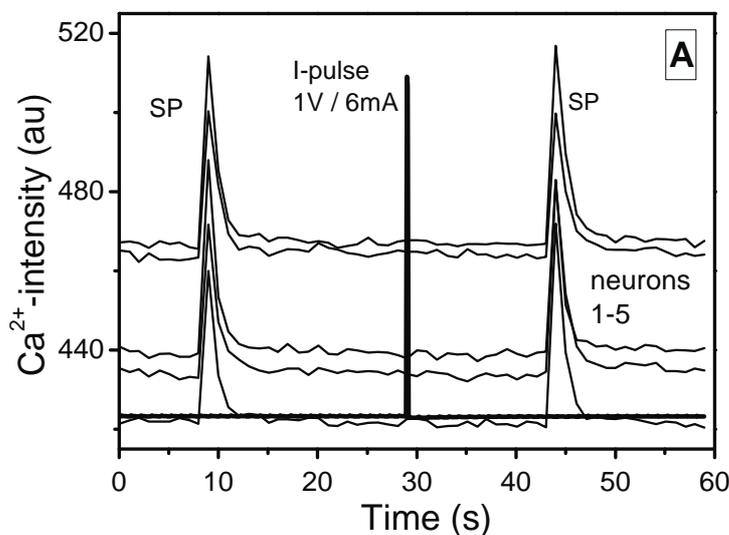


Figure 3: Cyclic voltammogram of the HEMT sensor in aCSF solution and in phosphate culture medium (PO) at 100 mV/s and the source as well as the drain contacts as the working electrode (A). Cyclic voltammogram of a bare gold finger as the working electrode in contact with the phosphate culture medium measured under the same conditions (B).

As can be seen in figure 2A, the open gate consists of the region between the source and drain contacts and two small stripes of the gold contacts (within the white frame) which are in direct contact with the cell processes and the culture medium. To rule out any electrolytic effects, we investigated the interface reactions between the culture medium and sensor by cyclic voltammetry. In figure 3 cyclic voltammograms (CV) of the HEMT structure are shown, in two different electrolytes, the artificial cerebrospinal fluid (aCSF) solution and the 0.1 M  $\text{KH}_2\text{PO}_4$  (PO) solution, which have been used as me-

dium cultures for neuron and yeast cells, respectively. For comparison, in figure 3 a CV of bare gold layers in finger contact arrangement (see also /13/) in contact with the PO culture mediums is also shown. The latter curve is characterized by appearance of a very well expressed oxidation peak and a strong reduction peak at about 0.48 V. These features are very similar to the anodic and cathodic peaks found in /14/ for flat gold films describing the oxidation and reduction of the gold electrode. In contrast, the CVs of HEMT in contact with both culture media do not show any significant faradaic processes in the investigated potential range. In the case of HEMT surface the capacitive behaviour dominates. This behaviour can be attributed to capacitive influence of the AlOx layer exposed to the investigated medium cultures /15/. In addition, the 2DEG induces positive charges to the HEMT surface wetted by the culture medium via field effect. In this case, it can be assumed that an electrical double layer is formed at the surface of the highly resistive AlGaN layer, which acts as an additional capacitance and contributes to the overall voltammetric response of the HEMT /16/. In conclusion, electrolytic reactions between the HEMT sensor surface and the culture medium are insignificant and do not contribute to the stimulation process of the neuron network.

For the stimulation of neurons with current pulses the source-drain voltage was enhanced from 0.1 V to a defined higher voltage with a pulse width of 100 ms and a pulse rate of rise of 1 ms after 28 s of the beginning of the measurements followed by a jump back to 0.1 V. The time dependence of the source-drain current was recorded together with the Fluo3  $\text{Ca}^{2+}$ -fluorescence signal of five neurons around the electrodes (see figure 2). In figure 4 the  $\text{Ca}^{2+}$ -fluorescence signals and the source-drain current pulses are drawn for three different stimulation intensities. All neurons showed spontaneous and synchronized activity ( $\text{Ca}^{2+}$ -fluorescence intensity peaks). For low current pulses ( $\leq 6$  mA, figure 4A) the neurons did not respond. Above a current of 7.5 mA (figure 4 B and C) fluorescence peaks are induced directly after the pulses verifying an electrical stimulation of the neurons.



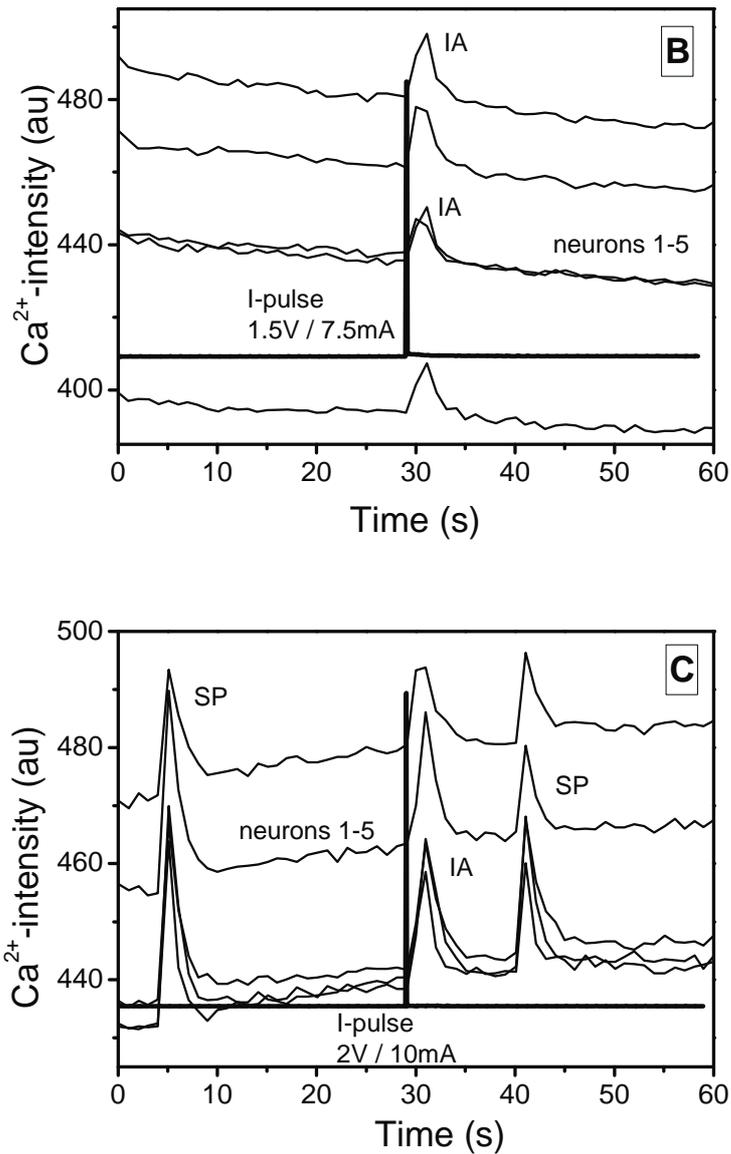


Figure 4: Time dependence of the  $\text{Ca}^{2+}$ -fluorescence intensity for 5 different single neurons and the corresponding time dependences of the current pulse at constant voltage which was applied after 28 s. The excitation conditions were varied by the voltage pulse amplitude: A - 1V pulse (6 mA); B - 1.5 V pulse (7.5 mA) and C - 2 V pulse (10 mA).

The voltage-current characteristics of stimulation pulses are plotted in figure 5 for two nominally equal neuronal cultures prepared under the same conditions and, as reference, for a dish with a-CSF medium only. Additionally, the IV-characteristic of the HEMT with a surface exposed to air is plotted as full line. All IV-characteristics are strongly linear proportional representing the resistance of the 2DEG channel in the range of about  $180 \Omega$  as the dominating part of the equivalent circuit. In both used neuronal cultures the variations of the voltage/current pulse amplitudes yield a threshold current determined as the minimal current at which the pulse stimulates the depolarization of the neurons.

Nevertheless, there is a difference between the threshold currents for neuron stimulation of the two cultures (1 and 2) demonstrating that the voltage pulse introduces different charge values into the culture medium, while the resistance remains nearly constant.

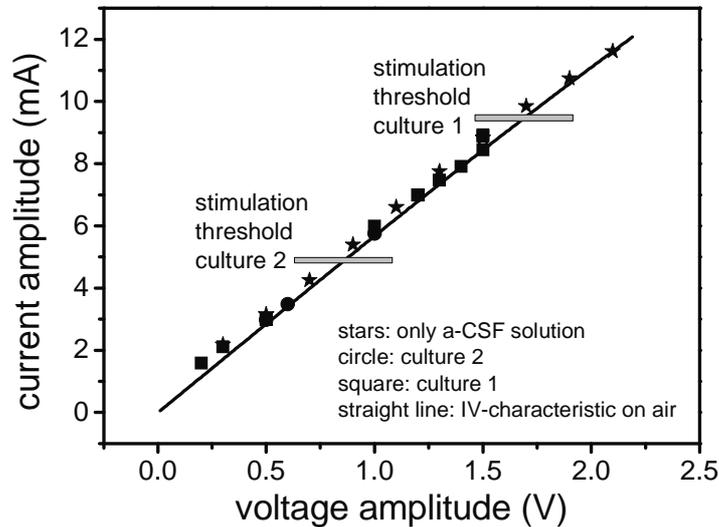


Figure 5: Current amplitude versus constant voltage pulse of two neuron cultures, an aCSF culture medium and a HEMT in air. The IV-characteristic shows a straight forward line but the threshold current amplitude for the excitation of the neurons differs strongly between both cultures.

To explain this discrepancy, we recorded the shapes of the pulses with dry electrodes, a-CSF solution and the neuron culture with the dye (figure 6). In air the current transients reproduce only the voltage pulse that is characteristic for Ohmic resistances. By using any solutions on the HEMT surface, after pulsing the current transient initially drops below the pre-pulse values and then returns to base line values. This behavior is characteristic for recharging of capacitances. There are two possible origins for the capacitances: (1) Current pulses inject positive charges into the neuron culture / HEMT interface by field effect above the AlGaN / top layer arrangement acting as a capacitance; (2) An additional current might flow from the contact feeds through the AlOx isolation layer, the HEMT surface-culture-interface and the through the culture medium to the other contact. In both cases, the source-drain current pulse induces a recharging of this complex network and the differences between the threshold currents can be explained mainly by different impedances of the neuron culture.

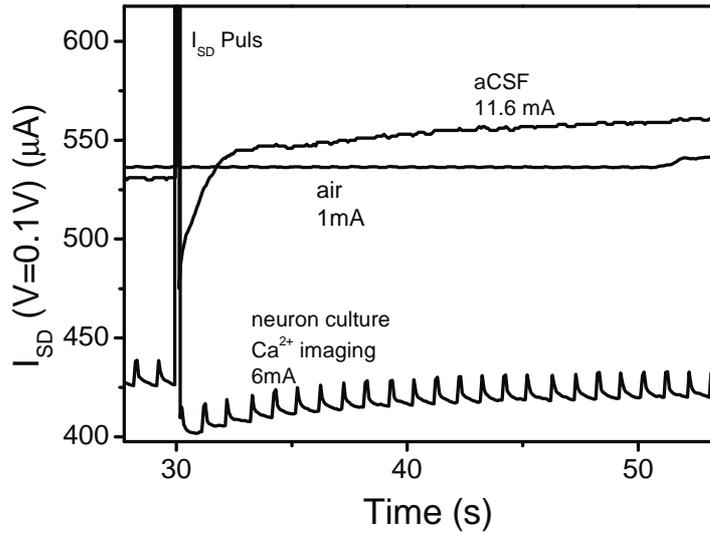


Figure 6: Time dependence of the current amplitude of the constant voltage pulse for different wetting of the HEMT surface. The oscillating spikes of the curve using neurons are caused by residual UV-light of the excitation lamp for Ca-imaging.

To test this assumption, we analyzed the impedances of four different HEMTs within one culture. The Bode plots in Fig. 7 show strong differences. The complex series capacitances and resistances measured for the HEMT numbers 1, 4, 7 and 9 (the numbers represent the location within the sensor shown in figure 1) are 509 nF and 28.9 $\Omega$ , 627 nF and 29.5 $\Omega$ , 98nF and 71.2 $\Omega$ , and 367nF and 37.1 $\Omega$ , respectively. The corresponding impedances at 1 kHz of these four HEMTs are dominated by the capacitive parts and yield 314  $\Omega$ , 255  $\Omega$ , 5.32 k $\Omega$  and 435  $\Omega$ , respectively. Assuming the AlGaN, GaN and isolation layers to be the same for all HEMTs the difference in impedances can only be explained by an inhomogeneous wetting of the contact surface or different conductivity of neuronal processes covering the electrode area. Since the growth of cell processes over the electrode area varies considerably between individual HEMTs this variation is most likely the source of the observed impedance differences and thus for the different stimulation conditions in the HEMT-neuron-interface.

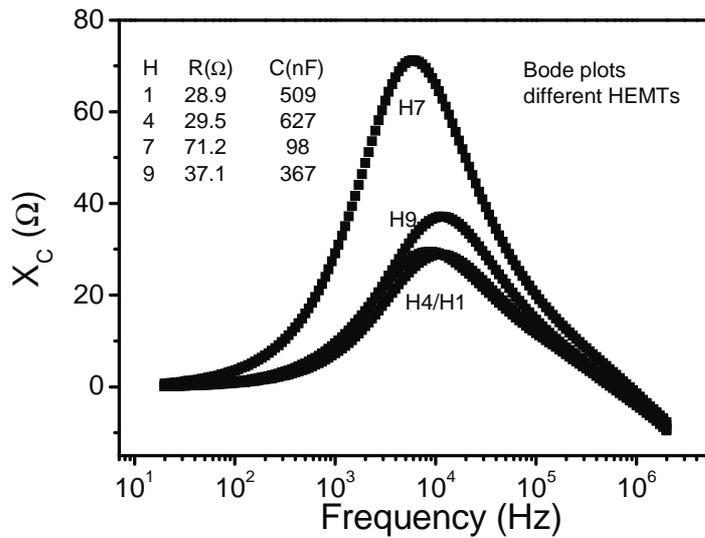


Figure 7: Imaginary part of the complex impedance in dependence of the frequency (Bode plot) for 4 different HEMTs onto the wafer sensor for the same neuron culture. The calculated resistances and capacitances assuming a parallel arrangement are shown.

#### 4. Summary

An AlGaIn/GaN wafer sensor containing 10 HEMTs concentrically arranged around the wafer centre was used for electrical stimulation of in situ cultured neurons. Neurons grown on the HEMTs show a spontaneously synchronized activity. A direct contact between the source-drain metal layer and the culture medium is avoided by an AlOx isolation and a small stripe of bare gold contact area show no detectable electrolytic reactions with the medium observed by cyclic voltametry. An unipolar voltage pulse was applied at the source-drain contacts of the HEMTs which in turn via the field effect induces a current in the neuron culture on top and leads to neuron activity. During the pulse process capacitances were recharged giving indications of the contribution of the AlGaIn and isolation layers between the 2DEG channel and the neuron culture. For different cultures there is a strong variation of the stimulation threshold current caused by different impedances of each neuronal culture on the stimulating HEMT under investigation. These different impedances can be explained by differences of the composition, the thickness and conductivity of the culture region.

#### Acknowledgement

This work was funded by the Federal State of Saxony-Anhalt and the “European Regional Development Fund” (ERDF 2007-2013).

#### References

- /1/ Opitz T, De Lima A D, Voigt T 2002 *J. Neurophysiol.* **88** 2196
- /2/ Baltz T, De Lima A D, Voigt T 2010 *Frontiers in Cellular Neuroscience*, doi: 10.3389/fncel.2010.00015
- /3/ Jimbo Y, Kasai N, Torimitsu K, Tateno T 2006 Chapter : MEA-based spike recording in cultured neuronal networks, in : *Frontiers in Biochip Technology*, Ed. Wan-Li Xing, Jing Cheng, Springer Science and Business Media Inc.
- /4/ Perelman Y, Ginosar R 2008 Chapter: MEA on Chip; in: *The neuron processor*, Springer Science and Business Media, Inc.
- /5/ Ingebrandt S, Offenhäuser A 2006 Chapter: Cell-Transistor Hybrid System; in: *Frontiers in Biochip Technology*, Ed. Wan-Li Xing, Jing Cheng; Springer Science and Business Media, Inc.
- /6/ Potter S M, Wagenaar DA, DeMarse T B 2006 Chapter: Closing the loop: stimulation feedback systems for embodied MEA cultures; in: “*Advances in Network electrophysiology: using MEAs*”, Ed. M.Taketani, M.Beudry, Springer Science and Business Media, Inc.
- /7/ Steinhoff G, Baur B, Ribbeck H-G, Wrobel G, Ingebrandt S, Offenhäuser A, Stutzmann M, Eickhoff M 2005 *Advances in Solid-State Physics* **45** 363
- /8/ Bayer M, Uhl C, Vogl P 2005 *J. Appl. Phys.* **97** 033703
- /9/ Witte H, Charpentier M, Warnke C, Mueller M, Guenther K-M, Dadgar A, Krost A 2010 *Phys. Stat. Sol. C*, **72** 464 – 467
- /10/ Pearton S J, Ren F, Wang Yu-Lin, Chu B H, Chen K H, Chang C Y, Lim W, Lin J, Norton D P 2010 *Progress in Mater. Sci.* **55** 1
- /11/ Steinhoff G, Baur B, Wrobel G, Ingebrandt S, Offenhäuser A, Dadgar A, Krost A, Stutzmann M, Eickhoff M 2005 *Appl. Phys. Lett.* **86** 033901
- /12/ Gebinova M, Cimalla I, Silveira L, Klett M, Lebedev V, Niebelschütz F, Ambacher O, Schober A 2009 Chapter: Response of nerve cell to inhibitor recorded by Aluminium-Gallium Nitride FET; in: *Sensors for Environment , Health and Security*; Ed. M.I.Baron; Springer Science and Business Media B.V.
- /13/ Reiher A, Warnke C, Radoch S, Witte H, Krtschil A, Mair T, Müller S C, Krost A 2006 *J. Bioenerg. Biomembr.* **38** 143
- /14/ Nogues C, Wanunu M 2004 *Surface Science* **573** L383
- /15/ Dang X D, Plieth W, Richter S, Plötner M, Fischer W-J 2008 *Phys. Stat. Sol (a)* **205** 626
- /16/ Podolska A, Kocan M, Cabezas A M., Wilson T D, Umana-Membreno G A, Nener B D, Parish G, Keller S, Mishra U K 2010 *Appl. Phys. Lett.* **97** 012108

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We used cyclic voltammetry to test whether the AlO<sub>x</sub> insulating layer completely prevents contact between the culture and the gold layer and to investigate the surface reactions of the open gate area of the HEMT which includes also two small stripes of bare gold contacts (see areas C in figure 2A). All measurements were made in a Pyrex electrochemical cell using potentiostat PGSTAT302 (Eco Chemie/Autolab, Netherlands); the interconnected source and drain contacts were used as the working electrode. A platinum wire represents the counter electrode and a saturated calomel electrode (SCE) the reference electrode. All potentials are applied versus the SCE.

For impedance measurements a precision LCR meter (HP 4284) in the frequency range between 20 Hz and 1 MHz at zero bias and with an AC measurement voltage of 30 mV is used. The source- drain current is measured with a source measure unit (Keithley 236) at a bias voltage of 0.1 V. For unipolar pulse experiments the voltage of the Source Measure Unit is switched from +0.1V up to +2V and back to +0.1 V with a pulse width of 100 ms and a pulse rising time of 1 ms.

To grow nerve cells directly on the HEMT sensors, a cultivation wall is formed by gluing a 20mm acrylic glass ring over the central sensor area. To improve cell adhesion and long term neuronal survival the cultivation area is coated with Poly-D-Lysine and purified astrocytes are plated inside the acrylic glass ring one week prior to the neuronal cells. After the glial cells are grown to confluence, neurons dissociated from embryonic rat cerebral cortex are plated on top of the glia feeder layer in a density of 800 cells/mm<sup>2</sup>. Neuronal cells are cultivated for up to 3 weeks in DMEM medium in a gassed incubator with 5% carbon dioxide content, humidity of 100% and a temperature of 37°C.

During the cultivation time neurons formed richly interconnected, electrically active networks. The spontaneous neuronal activity and their response to electric stimulation were accessed by optically recording the changes in the intracellular calcium concentration with the calcium sensitive dye Fluo-3AM./1/.

### 3. Results and Discussion

To test for biocompatibility neurons were plated and cultivated on the sensor surface and the development of spontaneous network activity was recorded by calcium imaging (figure 2). Neurons grew uniformly on the entire wafer surface including the contact zones, the region between the source drain and on the contact feeds (see areas C in figure 2A). We have qualitatively inspected the cell growth of all HEMTs on the wafer sensor and found no apparent preferences in the cellular distribution. Neurons showed spontaneous changes in their intracellular calcium concentration (figure 2B-D), corresponding to the firing of action potential bursts /1/.

As can be seen in figure 2A, the open gate consists of the region between the source and drain contacts and two small stripes of the gold contacts (within the white frame) which are in direct contact with the cell processes and the culture medium. To rule out any electrolytic effects, we investigated the interface reactions between the culture medium and sensor by cyclic voltammetry. In figure 3 cyclic voltammograms (CV) of the HEMT structure are shown, in two different electrolytes, the artificial cerebrospinal fluid (aCSF) solution and the 0.1 M  $\text{KH}_2\text{PO}_4$  (PO) solution, which have been used as medium cultures for neuron and yeast cells, respectively. For comparison, in figure 3 a CV of bare gold layers in finger contact arrangement (see also /13/) in contact with the PO culture mediums is also shown. The latter curve is characterized by appearance of a very well expressed oxidation peak and a strong reduction peak at about 0.48 V. These features are very similar to the anodic and cathodic peaks found in /14/ for flat gold films describing the oxidation and reduction of the gold electrode. In contrast, the CVs of HEMT in contact with both culture media do not show any significant faradaic processes in the investigated potential range. In the case of HEMT surface the capacitive behaviour dominates. This behaviour can be attributed to capacitive influence of the  $\text{AlOx}$  layer exposed to the investigated medium cultures /15/. In addition, the 2DEG induces positive charges to the HEMT surface wetted by the culture medium via field effect. In this case, it can be assumed that an electrical double layer is formed at the surface of the highly resistive  $\text{AlGaN}$  layer, which acts as an additional capacitance and contributes to the overall voltammetric response of the HEMT /16/. In conclusion, electrolytic reactions between the HEMT sensor surface and the culture medium are insignificant and do not contribute to the stimulation process of the neuron network.

For the stimulation of neurons with current pulses the source-drain voltage was enhanced from 0.1 V to a defined higher voltage with a pulse width of 100 ms and a pulse rate of rise of 1 ms after 28 s of the beginning of the measurements followed by a jump back to 0.1 V. The time dependence of the source-drain current was recorded together with the Fluo3  $\text{Ca}^{2+}$ -fluorescence signal of five neurons around the electrodes (see figure 2). In figure 4 the  $\text{Ca}^{2+}$ -fluorescence signals and the source-drain current pulses are drawn for three different stimulation intensities. All neurons showed spontaneous and synchronized activity ( $\text{Ca}^{2+}$ -fluorescence intensity peaks). For low current pulses ( $\leq 6$  mA, figure 4A) the neurons did not respond. Above a current of 7.5 mA (figure 4 B and C) fluorescence peaks are induced directly after the pulses verifying an electrical stimulation of the neurons.

The voltage-current characteristics of stimulation pulses are plotted in figure 5 for two nominally equal neuronal cultures prepared under the same conditions and, as reference, for a dish with a-CSF medium only. Additionally, the IV-characteristic of the HEMT with a surface exposed to air is plotted as full line. All IV-characteristics are strongly linear proportional representing the resistance of the 2DEG channel in the range of about  $180 \Omega$  as the dominating part of the equivalent circuit. In both used neuronal cultures the variations of the voltage/current pulse amplitudes yield a threshold current determined as the minimal current at which the pulse stimulates the depolarization of the neurons. Nevertheless, there is a difference between the threshold currents for neuron stimulation of the two cultures (1 and 2) demonstrating that the voltage pulse introduces different charge values into the culture medium, while the resistance remains nearly constant.

To explain this discrepancy, we recorded the shapes of the pulses with dry electrodes, a-CSF solution and the neuron culture with the dye (figure 6). In air the current transients reproduce only the voltage pulse that is characteristic for Ohmic resistances. By using any solutions on the HEMT surface, after pulsing the current transient initially drops below the pre-pulse values and then returns to base line values. This behavior is characteristic for recharging of capacitances. There are two possible origins for the capacitances: (1) Current pulses inject positive charges into the neuron culture / HEMT interface by field effect above the AlGaN / top layer arrangement acting as a capacitance; (2) An additional current might flow from the contact feeds through the AlOx isolation layer, the HEMT surface-culture-interface and the through the culture medium to the other contact. In both cases, the source-drain current pulse induces a recharging of this complex network and the differences between the threshold currents can be explained mainly by different impedances of the neuron culture.

To test this assumption, we analyzed the impedances of four different HEMTs within one culture. The Bode plots in Fig. 7 show strong differences. The complex series capacitances and resistances measured for the HEMT numbers 1, 4, 7 and 9 (the numbers represent the location within the sensor shown in figure 1) are  $509 \text{ nF}$  and  $28.9 \Omega$ ,  $627 \text{ nF}$  and  $29.5 \Omega$ ,  $98 \text{ nF}$  and  $71.2 \Omega$ , and  $367 \text{ nF}$  and  $37.1 \Omega$ , respectively. The corresponding impedances at  $1 \text{ kHz}$  of these four HEMTs are dominated by the capacitive parts and yield  $314 \Omega$ ,  $255 \Omega$ ,  $5.32 \text{ k}\Omega$  and  $435 \Omega$ , respectively. Assuming the AlGaN, GaN and isolation layers to be the same for all HEMTs the difference in impedances can only be explained by an inhomogeneous wetting of the contact surface or different conductivity of neuronal processes covering the electrode area. Since the growth of cell processes over the electrode area varies considerably between individual HEMTs this variation is most likely the source of the observed impedance differences and thus for the different stimulation conditions in the HEMT-neuron-interface.

#### **4. Summary**

An AlGaN/GaN wafer sensor containing 10 HEMTs concentrically arranged around the wafer centre was used for electrical stimulation of in situ cultured neurons. Neurons grown on the HEMTs show a

spontaneously synchronized activity. A direct contact between the source-drain metal layer and the culture medium is avoided by an AlO<sub>x</sub> isolation and a small stripe of bare gold contact area show no detectable electrolytic reactions with the medium observed by cyclic voltametry. An unipolar voltage pulse was applied at the source-drain contacts of the HEMTs which in turn via the field effect induces a current in the neuron culture on top and leads to neuron activity. During the pulse process capacitances were recharged giving indications of the contribution of the AlGa<sub>N</sub> and isolation layers between the 2DEG channel and the neuron culture. For different cultures there is a strong variation of the stimulation threshold current caused by different impedances of each neuronal culture on the stimulating HEMT under investigation. These different impedances can be explained by differences of the composition, the thickness and conductivity of the culture region.

### Acknowledgement

This work was funded by the Federal State of Saxony-Anhalt and the “European Regional Development Fund” (ERDF 2007-2013).

### References

- /1/ Opitz T, De Lima A D, Voigt T 2002 *J. Neurophysiol.* **88** 2196
- /2/ Baltz T, De Lima A D, Voigt T 2010 *Frontiers in Cellular Neuroscience*, doi: 10.3389/fncel.2010.00015
- /3/ Jimbo Y, Kasai N, Torimitsu K, Tateno T 2006 Chapter : MEA-based spike recording in cultured neuronal networks, in : *Frontiers in Biochip Technology*, Ed. Wan-Li Xing, Jing Cheng, Springer Science and Business Media Inc.
- /4/ Perelman Y, Ginosar R 2008 Chapter: MEA on Chip; in: *The neuron processor*, Springer Science and Business Media, Inc.
- /5/ Ingebrandt S, Offenhäuser A 2006 Chapter: Cell-Transistor Hybrid System; in: *Frontiers in Biochip Technology*, Ed. Wan-Li Xing, Jing Cheng; Springer Science and Business Media, Inc.
- /6/ Potter S M, Wagenaar DA, DeMarse T B 2006 Chapter: Closing the loop: stimulation feedback systems for embodied MEA cultures; in: “*Advances in Network electrophysiology: using MEAs*”, Ed. M.Taketani, M.Beudry, Springer Science and Business Media, Inc.
- /7/ Steinhoff G, Baur B, Ribbeck H-G, Wrobel G, Ingebrandt S, Offenhäuser A, Stutzmann M, Eickhoff M 2005 *Advances in Solid-State Physics* **45** 363
- /8/ Bayer M, Uhl C, Vogl P 2005 *J. Appl. Phys.* **97** 033703
- /9/ Witte H, Charpentier M, Warnke C, Mueller M, Guenther K-M, Dadgar A, Krost A 2010 *Phys. Stat. Sol. C*, **72** 464 – 467

- /10/ Pearton S J, Ren F, Wang Yu-Lin, Chu B H, Chen K H, Chang C Y, Lim W, Lin J, Norton D P 2010 Progress in Mater. Sci. **55** 1
- /11/ Steinhoff G, Baur B, Wrobel G, Ingebrandt S, Offenhasser A, Dadgar A, Krost A, Stutzmann M, Eickhoff M 2005 Appl. Phys. Lett. **86** 033901
- /12/ Gebinova M, Cimalla I, Silveira L, Klett M, Lebedev V, Niebelschutz F, Ambacher O, Schober A 2009 Chapter: Response of nerve cell to inhibitor recorded by Aluminium-Gallium Nitride FET; in: Sensors for Enviroment , Health and Security; Ed. M.I.Baron; Sringer Science and Business Media B.V.
- /13/ Reiher A, Warnke C, Radoch S, Witte H, Krtschil A, Mair T, Muller S C, Krost A 2006 J. Bioenerg. Biomembr. **38** 143
- /14/ Nogues C, Wanunu M 2004 Surface Science **573** L383
- /15/ Dang X D, Plieth W, Richter S, Plotner M, Fischer W-J 2008 Phys. Stat. Sol (a) **205** 626
- /16/ Podolska A, Kocan M, Cabezas A M., Wilson T D, Umana-Membreno G A, Nener B D, Parish G, Keller S, Mishra U K 2010 Appl. Phys. Lett. **97** 012108

## Figure captions

Figure 1: Picture (left) and scheme (right) of the wafer sensor contains 10 HEMT structures on a two inch sapphire wafer. The wafer sensor is glued in a petri disc and into a holder involving the solder posts.

Figure 2: (A) Neurons grown on a HEMT structure. The source (S) and drain (D) contacts as well as the contacts feeds can be seen. The white frame represents the open gate area (G) and the bare gold regions (C). (B – D) Calcium imaging of spontaneous network activity of the field shown in (A): 2s before (B) during (C) and 2s after (D) a synchronous depolarization in five neurons. The spontaneous depolarization of the neurons shows that they are viable and electric active.

Figure 3: Cyclic voltammogram of the HEMT sensor in aCSF solution and in phosphate culture medium (PO) at 100 mV/s and the source as well as the drain contacts as the working electrode (A). Cyclic voltammogram of a bare gold finger as the working electrode in contact with the phosphate culture medium measured under the same conditions (B).

Figure 4: Time dependence of the  $\text{Ca}^{2+}$ -fluorescence intensity for 5 different single neurons and the corresponding time dependences of the current pulse at constant voltage which was applied after 28 s. The excitation conditions were varied by the voltage pulse amplitude: A - 1V pulse (6 mA); B - 1.5 V pulse (7.5 mA) and C - 2 V pulse (10 mA).

Figure 5: Current amplitude versus constant voltage pulse of two neuron cultures, an aCSF culture medium and a HEMT in air. The IV-characteristic shows a straight forward line but the threshold current amplitude for the excitation of the neurons differs strongly between both cultures.

Figure 6: Time dependence of the current amplitude of the constant voltage pulse for different wetting of the HEMT surface. The oscillating spikes of the curve using neurons are caused by residual UV-light of the excitation lamp for Ca-imaging.

Figure 7: Imaginary part of the complex impedance in dependence of the frequency (Bode plot) for 4 different HEMTs onto the wafer sensor for the same neuron culture. The calculated resistances and capacitances assuming a parallel arrangement are shown.