Improved Functionalization of InGaN Sensor Structures

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This report presents continued work on the functionalization of InGaN quantum well structures for biochemical sensing. Previous work of our group had shown functionalization of GaN surfaces for selective ferritin binding via microstamping. The contrast of fluorescence micrographs was low due to strong unspecific binding of the ferritin complex to non-functionalized InGaN areas. The microstamping technique is improved to achieve maximal contrast and thus lowest unspecific binding. An alternative techique of microdrop casting is introduced to encounter possible drawbacks of microstamping.

1. Introduction

Since their breakthrough over the last decades gallium nitride (GaN) and its alloys indium gallium nitride (InGaN) and aluminium gallium nitride (AlGaN) have been established as the gold standard for short wavelength visible light emitting diodes (LEDs). Because of its high bandgap, it has also gained increased interest as a base material for high-power electronics [1]. Due to its chemical stability and temperature resistance [2, 3] it is also well-suited for extreme environments as has been shown by various groups [4]. In biological environments, chemical inertness is a crucial parameter to minimize interaction with the respective specimens like cells or enzymes. It has been shown by our group that biochemical sensors can be made of GaN with a purely optical readout by making use of the quantum-confined Stark effect (QCSE) [5]. One big advantage of this approach is the possibility to remotely excite and analyze the structure to make use of InGaN's high stability. The photoluminescence (PL) of InGaN quantum wells (QWs) located closely to the surface of a GaN bulk layer shows sensitivity to surface-related potential changes. When (macro-)molecules are adsorbed on the surface, they eventually give rise to surface charges and thus create a zone of near-surface band-bending. The QWs are affected by the resulting field and their luminescence spectrum is changed in intensity and energy position (QCSE). This shift can be used to evaluate the potential change on the surface of the GaN layer.

In sensor theory, selectivity is just as important as is the sensitivity of a system. The detection process of the above mentioned effect is highly non-selective, as it is purely electronic. Various molecules may adsorb to GaN with different affinities but the change of the sensor signal cannot be attributed to a single substance. In order to increase the selectivity, a functionalization layer can be attached to the surface of the sensor. Different standard techniques exist in biochemistry, but each one has to be established for a new

material to work in the most effective way. For GaN, various groups have shown different functionalization techniques [6, 7].

In previous work of our group, the functionalization of InGaN QWs has been pursued, and a preferred attachment of ferritin molecules to functionalized areas could be demonstrated. The interaction chosen for our sensor system is the biotin-streptavidin interaction, which is one of the strongest, non-covalent reactions in biochemistry (dissociation constant $K_\mathrm{d} \approx 4 \cdot 10^{14}\,\mathrm{mol/l}$) [8]. Biotin (also known as vitamin B7) is involved in the synthesis of fatty acids and for the transformation of certain non-carbohydrates into sugar (gluconeogenesis). However for the first experiments, the contrast between functionalized and non-functionalized surfaces was low due to strong unspecific binding to the surface. In this report, our studies to improve the situation are presented.

2. Basic Functionalization Procedure

In previous work, functionalization has been shown by our project group [9], with position-selective binding of a ferritin-biotin-rhodamine (FBR) complex being demonstrated. The process has several steps as will be discussed below (compare Fig. 1).

In order to enable a binding of our analyte (ferritin), the first step is to create OH-groups on the GaN surface. This can be done by either annealing the sample in an oxygen plasma asher for 30–60 min at 100 W or by dipping it into a solution of hydrogen peroxide with sulfuric acid ($H_2O_2 : H_2SO_4$ in 1:3) for 10 min (piranha solution). The OH-groups then enable covalent binding of the PEG-silane-biotin complex with its Si-group. This is done in a way to create areas of silane functionalization and areas without. This way, in a later sensing analysis, the response of the system can be analyzed with respect to different regions to compare the shift introduced by the biomolecules. After incubating the silane complex for 15 min, streptavidin (strp) is added to the surface and incubated for 30–60 min. Biotin fits into one of four binding pockets of strp, where it is (among others) fixed with hydrogen bonds and van der Waals interaction [10]. Strp has 4 binding sites for biotin, which enables the last step of the process: Attaching the FBR-complex to the remaining binding sites of strp (incubation time: 30–60 min). After each subsequent step, thorough washing is performed, to reduce non-specific binding of the complexes to the GaN surface.

After deposition, the sensor sample is analyzed in a fluorescent microscope. The FBR-complex is visible due to rhodamine, which is a fluorescent dye commonly used in biochemistry. After analysis of the pattern, the sample can be put into our project's automated micro-PL setup. With this setup, a PL map can be created, where every pixel corresponds to a measured PL spectrum. The emission of the QW is analyzed with a center of mass method to determine the center wavelength. In order to be able to refer both methods to one another, gold markers are deposited prior to all experiments on the GaN structure. This way, a reference system is created to compare both techniques at the same positions.

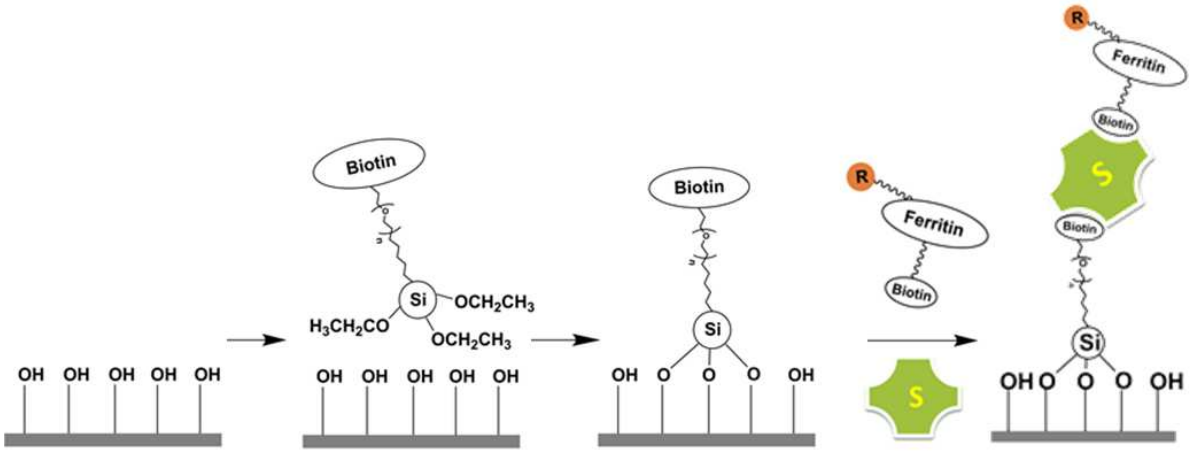

Fig. 1: The functionalization process shown in steps: The hydroxilated GaN surface enables covalent binding of the PEG-silane-complex. The second step creates a binding between strp (S) and biotin of the PEG-silane. In the last step, the same interaction is used for binding the FBR-complex to the free binding sites of strp. The dark dot (R) indicates rhodamine.

3. Surface Pattering Techniques

3.1 Microcontact printing

The technique used in most of our experiments is microstamping: A silicon wafer structured by photolithography is used to mold polydimethylsiloxane (PDMS) polymer into a high-low-pattern. A holder is attached, and after drying, the polymer can be carefully released from the wafer to create the inverse stamping pattern of the wafer. The stamp is then vapor-loaded with the respective silane in a glass cylinder for 30 min (compare [9]). Thin layers of silane can thus be expected to be deposited on the stamp and thus later on the substrates. For the stamping process itself, a dip-coating machine is used, which provides a constant up- or down-movement. The force which is applied to the structure is regulated with a spring-dampened base-plate, on which the sample is fixed during the process. This way, the time of lowering the stamp is proportional to the downward force of the stamp. Stamping provides a very directed deposition of the silanes onto GaN and incubation after stamping is not necessary anymore. On the other hand, the time required for loading the stamp with silane takes 30–60 min, and the PDMS material shows a soaking effect, which deteriorates the stamp after few uses and requires new stamps to be made.

3.2 Microdrop casting

The second technique used in this work is the technique of microdrop casting: A commercial, piezo-driven nozzle with a diameter of few micrometers shoots drops of ≈ 200 pl at a repetition rate of up to 1 kHz. The deposited amount can be regulated precisely by shooting several drops on one spot before moving to the next position. The system enables homogeneous and repeatable deposition rates and well-defined molecule amounts

due to possible dilution of the used solution. The main drawback of this technique is possible clogging of the nozzle with viscous molecule solutions and thus a potential timely nozzle cleaning process before every run (acetone is flushed through the system to dissolve all contaminants and to release possible clogs). After deposition and incubation of silane molecules on GaN, excess material has to be removed with supersonication for at least 1 min in deionized (DI) water.

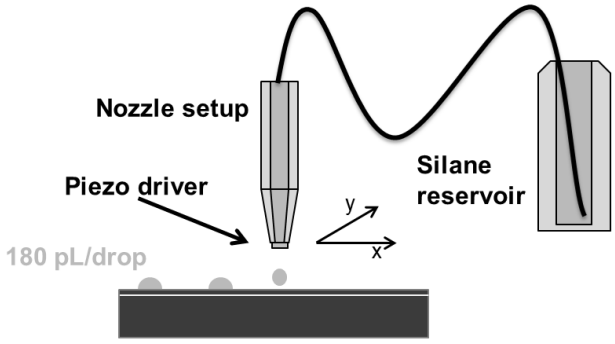

Fig. 2: Schematic of the microdrop setup: A piezo-driven nozzle shoots $\approx$ 200 pl/drop at a repetition rate of up to 1 kHz. A reservoir of $\approx$ 2 ml is connected with a tube to enable continuous operation for up to three hours. When mounted into the automated micro-PL setup, the drops can be deposited in different amounts per spot, as the system moves after each deposition.

4. Sensing Experiments

4.1 Sensing of stamped structures

As mentioned in the introduction, first functionalization results have been obtained for GaN by our project group [9]. The system used in this previous work made use of covalent bindings. 3-mercaptopropyltrimethoxysilane (MPTMS) binds to the OH-groups (hydroxy) of a prepared GaN surface. MPTMS has a functional SH-group (thiole) which binds covalently to the maleimide-group of ferritin-maleimide-complex. A big drawback of this technique is the lack of possible regeneration of the sensor surface for later experiments. In order to break the strong covalent bonding between the analyte (ferritin) and the sensor, aggressive chemistry would have to be used which also removes MPTMS. A solution to this problem is the use of a weaker interaction. The biotin-streptavidin interaction can be removed with a nonionic aqueous solution at 70 °C [8], but otherwise is very strong and stable. In order to reduce unspecific binding, the concentration of the FBR solution was reduced (previously 5 mg/ml, now 0.02 mg/ml), and a more thorough washing process was established. All constituents in the newly used process are water soluble, enabling efficient washing with Milli-Q water in an ultrasonic bath. Subsequent sonication after every step highly improved the contrast of stamped structures (compare Fig. 3). The next step was to evaluate the stamped structures via the sensor signal itself. For this, the automated micro-PL setup was used to scan over a previously selected area, where stamps as well as the gold markers could be seen (see Sect. 2). The area was scanned in steps of 50 µm and a total area of $700 \times 700\,\mu m^2$ was analyzed. For each step, a PL-spectrum was recorded. The center wavelength and the intensity of the PL signal is analyzed automatically. The corresponding results can be seen in Fig. 4: While

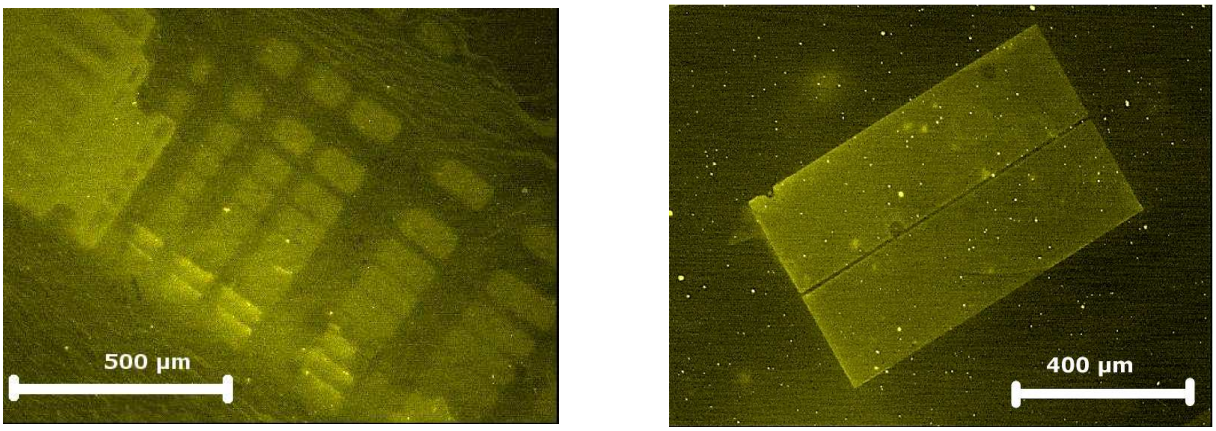

Fig. 3: Comparison between previous stamping results (left) and optimized structures with the biotin-strp interaction (right) in fluorescence micrographs: Strong nonspecific binding can be observed for the old method, the contrast between stamped areas and the GaN surface is low. For the biotin-strp, the edges of the stamped areas are clearly visible and even fine details (the 15 µm groove between both stamped bars) can be resolved.

the stamped pattern is clearly visibile alongside the gold marker in the fluorescence micrograph, the same structure cannot be seen as a sensor response in the PL-map. The color scale of the map is set according to the PL peak center energy: high (medium grey), medium (bright grey), and low (dark grey) energies. An eventual shift can be observed in the area where the groove between both stamped bars is located (double arrow). This might be attributed to a local accumulation of molecules at the groove. As the molecules are probably attached in densities close to a monolayer, the sensor response might be too small for the non-optimized sensor yet. For this reason, an alternative functionalization method via microdrops was introduced for potentially higher molecule concentrations on the sensor surface.

4.2 Functionalization of microdrops on GaN

The micropdrop setup was built to be mounted into the micro-PL setup for automated dropcasting. In this way, the sensor sample can be patterned similar to the microstamp technique. The volume of one drop is small enough to enable structure sizes of $\approx 200\,\mu m$, thus being similar to the stamped structures. In Fig. 5 an example of dropcasting on glass is shown: via a camera, the dropcasting process can be evaluated and adjusted ad-hoc. Glass was chosen as a cheap substrate for the first optimization eperiments with this new technique, as for InGaN, the glass parameters can be used and then improved. Different amounts of drops were investigated per spot. After every dropcasting procedure, the silane drops were incubated for 15 min at 40 °C and subsequently excess silane was removed by supersonication in Milli-Q water for 1 min. In the following incubation processes for streptavidin and FBR (both 0.02 mg/ml for 30 min), a humidic chamber was used to minimize drop evaporation and thus inhibit drying inhomogeneities. After strp incubation, sonication in buffer solution[2] was used to remove excess streptavidin. After

[2] 10 mM PBS (pH 7.4), 0.3 M NaCl, 0.1 % Tween20

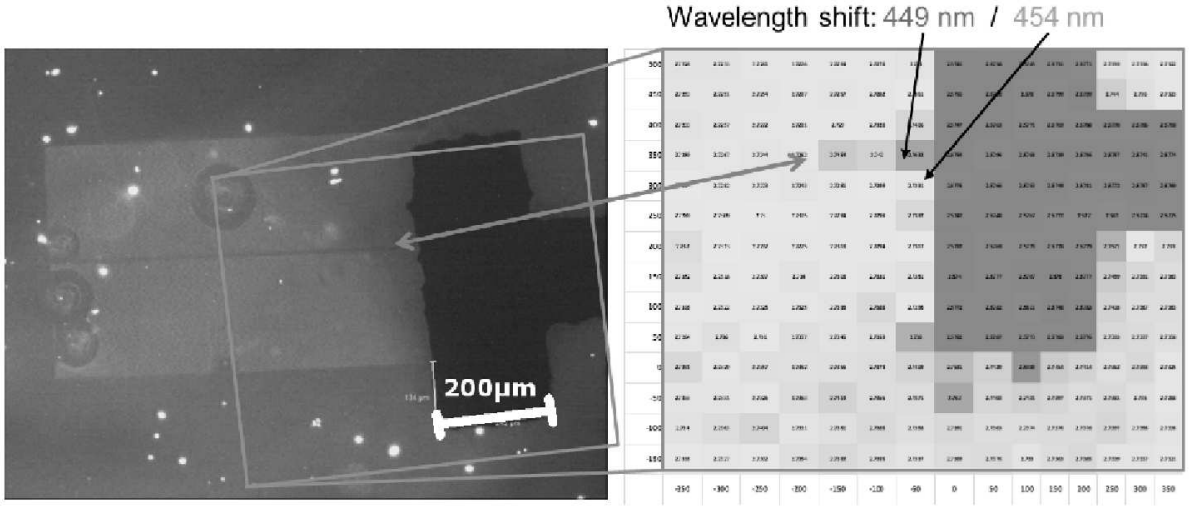

Fig. 4: A functionalized sensor area was investigated by fluorescence microscopy (left): The double-bar structure is clearly visible with high resolution and contrast. When analyzed in the micro-PL setup, there is no clear sensor response visible (right). The colorscale is set according to the PL peak center energy: high (medium grey), medium (bright grey), and low (dark grey) energies. An energy shift can be observed at the position of the groove between both stamped bars (double arrow).

the FBR incubation, the sample was rinsed in Milli-Q water 5–10 times. The optimum drop amount was found to be 10 which translates into a volume of $\approx 2\,\mathrm{nl}$. Below this volume, the drops evaporate too fast for efficient incubation, and above, drops show reduced homogeneity in the observed pattern. In a next step, these procedures will be transfered to our InGaN sensor structures.

5. Conclusion and Outlook

In this work, different approaches for InGaN functionalization have been investigated. The stamping technique was optimized for low unspecific binding and thus high contrast in fluorescent micrographs. One drawback of the stamping procedure is the degradation of the stamp itself and the not well-defined amount of silane molecules on the stamp and thus on the sensor. In the micro-PL setup, no explicit response could be found. This might be attributed to the incomplete coverage of the monolayer and thus very low molecule concentration, or to the sensitivity of the sensor being too low. As reported in previous work from this group, simulations have been performed to improve the sensitivity of the sensor structure. With new structures being grown and functionalized an effective response might be received in the future.

In the second part, a new functionalization technique is introduced: microdrop casting. This technique enables better control of the actual amount of molecules being deposited on the sensor surface and was proven to work effectively on glass substrate. Referring to our experience with stamping, it can be followed that the parameters for ideal functionaliza-

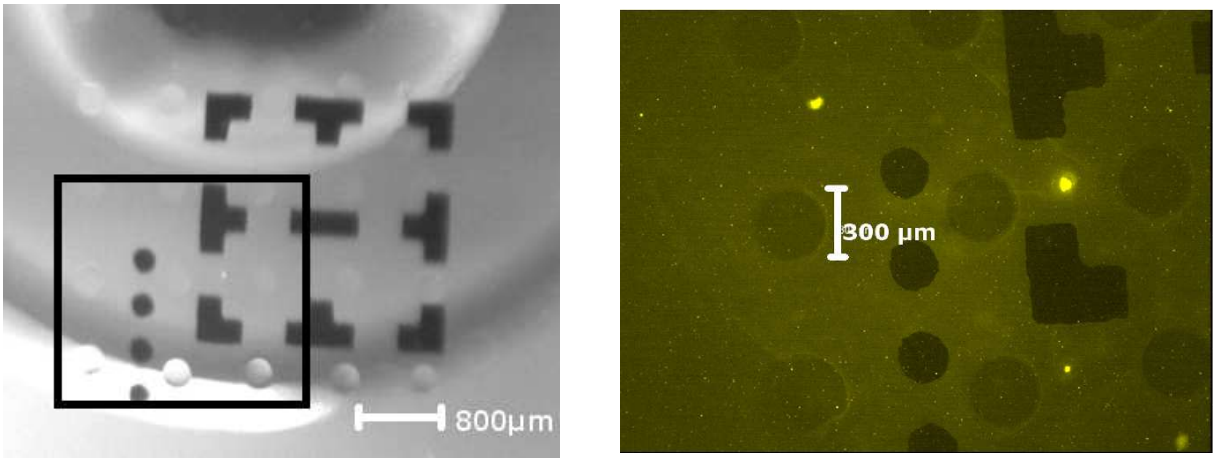

Fig. 5: The dropcasting process can be monitored within the automated micro-PL setup and then related to FL- and PL-measurements (left). The drops can be seen best in the bottom left corner of the black square. The same area as seen in the FL-microscope (right): Drops can be clearly seen alongside the gold marks. They show an inverse behaviour, such that they seem darker than the surounding surface. This was due to non optimized incubation steps and was resolved in later experiments.

tion should not differ strongly for GaN and thus can be adapted. Subsequently, sensing experiments will be performed on these new structures. For live sensing, microfluidic channels can be introduced, which enable measurement of the various functionalization steps. This way a clearer understanding can be drawn of how the various binding steps affect the sensor response.

Acknowledgment

The work presented in this article is a close collaboration of our IOB project group constisting of my fellow PhD colleagues F. Huber and N. Naskar, and the project leaders Prof. K. Thonke, Prof. T. Weil, and my supervisor Prof. F. Scholz. Most results have been obtained from experiments performed by a group of two or more of the PhD students. Financial support by the Baden-Württemberg Stiftung gGmbH within the project "Intelligente optoelektronische Biosensoren" is gratefully acknowledged.

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