Selective Functionalization of GaInN Quantum Well Surfaces for Applications in Biosensing

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In this work, chemical functionalization of the surface of gallium indium nitride (GaInN) quantum well structures is performed aiming to study biosensing of the iron-storage molecule ferritin. In order to enable local investigations of ferritin molecules, a local functionalization of the group III-nitride surface with silane molecules is performed. Our studies demonstrate a preferred attachment of ferritin molecules on the functionalized quantum well surface.

1. Introduction

Besides prominent applications in general lighting and lasing, chemical sensing based on III-nitride semiconductors finds increasing interest [1–5]. Group III-nitrides provide a very stable [6] and biocompatible [5] material system with excellent optoelectronic properties. Today, biosensing methods are frequently based on flourescent labels which can be selectively linked to biomolecules [7] and enable subsequent optical investigations down to the microscopic scale [7, 8].

However, fluorescent dyes are often reported to suffer from photobleaching effects [9, 10] and weak light intensity [10] which limits applications in biosensing [7, 9, 11]. Moreover, fluorescent molecules are typically not selective to specific molecular properties such as the iron-load of ferritin molecules [12, 13]. Hence, new label-free sensing approaches are of interest for next generation optical biosensing. Ferritin molecules play a key role in regulating the iron status of the human body [12]. Within their spherical cavity, up to 4500 iron atoms can be reversibly stored [12]. Besides the molecular concentration of ferritin in human blood, the iron-load is reported as a superior biomarker [13].

In our recent studies, a new approach for sensing ferritin bound iron is demonstrated applying polar GaInN quantum wells as optochemical transducers [14]. Ferritin and apoferritin molecules immobilized onto the GaN semiconductor surface cause spectral shifts of the polar GaInN quantum well photoluminescence [14]. Apoferritin corresponds to ferritin molecules without iron-load. Spectral shifts are associated to changes of the quantum confined Stark effect (QCSE) present in polar GaInN quantum wells.

In this study, a local functionalization of GaInN quantum well structures for selective binding of ferritin molecules is studied. Hence, local photoluminescence changes caused by adsorbed ferritin molecules can be investigated in micro-photoluminescence spectroscopy.

2. Key-Lock Principle

In order to enable local investigations of (apo)ferritin molecules, a chemical functionalization of the III-nitride semiconductor surface with silane molecules is studied. Both, the semiconductor surface and (apo)ferritin molecules are functionalized with molecules which obey very specific binding properties. By using a key-lock principle, (apo)ferritin molecules can be locally attached to the III-nitride semiconductor surface. A schematic illustration of the applied key-lock principle for selective attachment of ferritin on GaN is given in Fig. 1.

In this work, a local surface functionalization with 3-mercaptopropyltrimethoxysilane (MPTMS) is studied. Silane molecules bind to OH groups present on the hydroxylated GaN surface and obey a functional SH group. A functional maleimide group can be attached to (apo)ferritin. The interaction between the functional SH group of MPTMS and the maleimide group is highly specific [16]. Ferritin molecules with functional maleimide group selectively bind to the SH group present on the silanized semiconductor surface. Optionally, fluorescent rhodamine dyes can be attached to (apo)ferritin in order to allow fluorescence microscopic investigations for cross-checking the surface functionalization. In the target sensor design, local changes of the GaInN quantum well photoluminescence shall be used for sensing the ferritin molecules as discussed in [14].

Two different approaches for local silanization of group III-nitrides are investigated. First, selective stamping of silanes onto the semiconductor surface is performed. Polydimethylsiloxane (PDMS) stamps are realized using structured silicon wafers as templates. A SU-8 2000 photoresist is structured on the silicon wafer using conventional optical lithography. The viscous PDMS is molded on the structured wafer and hardened for several hours. Subsequently, the elastic PDMS stamp can be removed from the substrate and mounted on a step motor based setup which allows a controlled stamping of different surfaces with silanes. The detailed stamping sequence is given in Fig. 2.

In order to exclude silanization of nominally unpatterned areas, a second approach based on the selective evaporation of silanes onto the semiconductor surface is applied using a shadow mask. MPTMS is evaporated onto the uncovered areas of the sample. Subsequently, the mask is removed from the sample surface and the template is rinsed with toluene to remove unbound silanes. In contrast to the micro-contact patterning, an improved selectivity is expected with less silane molecules in nominally unpatterned areas.

Polar GaInN quantum well structures are realized on sapphire wafers in a commercial Aixtron AIX200/RF metal organic vapor phase epitaxy (MOVPE) reactor using ammonia (NH_3), trimethylgallium (TMGa), trimethylaluminum (TMAl), triethylgallium (TEGa), and trimethylindium (TMIn) as precursors. First, a thin oxygen-doped aluminum nitride (AlN) nucleation layer is realized, followed by a Ga-polar GaN buffer layer. GaInN quantum wells with a thickness of 3 nm, 7 nm GaN barrier thickness, and a GaN cap layer with a thickness of about 7 nm are realized. Before deposition of the silane molecules, the semiconductor surface is hydroxylated using a mixture of sulfuric acid and hydrogen peroxide.

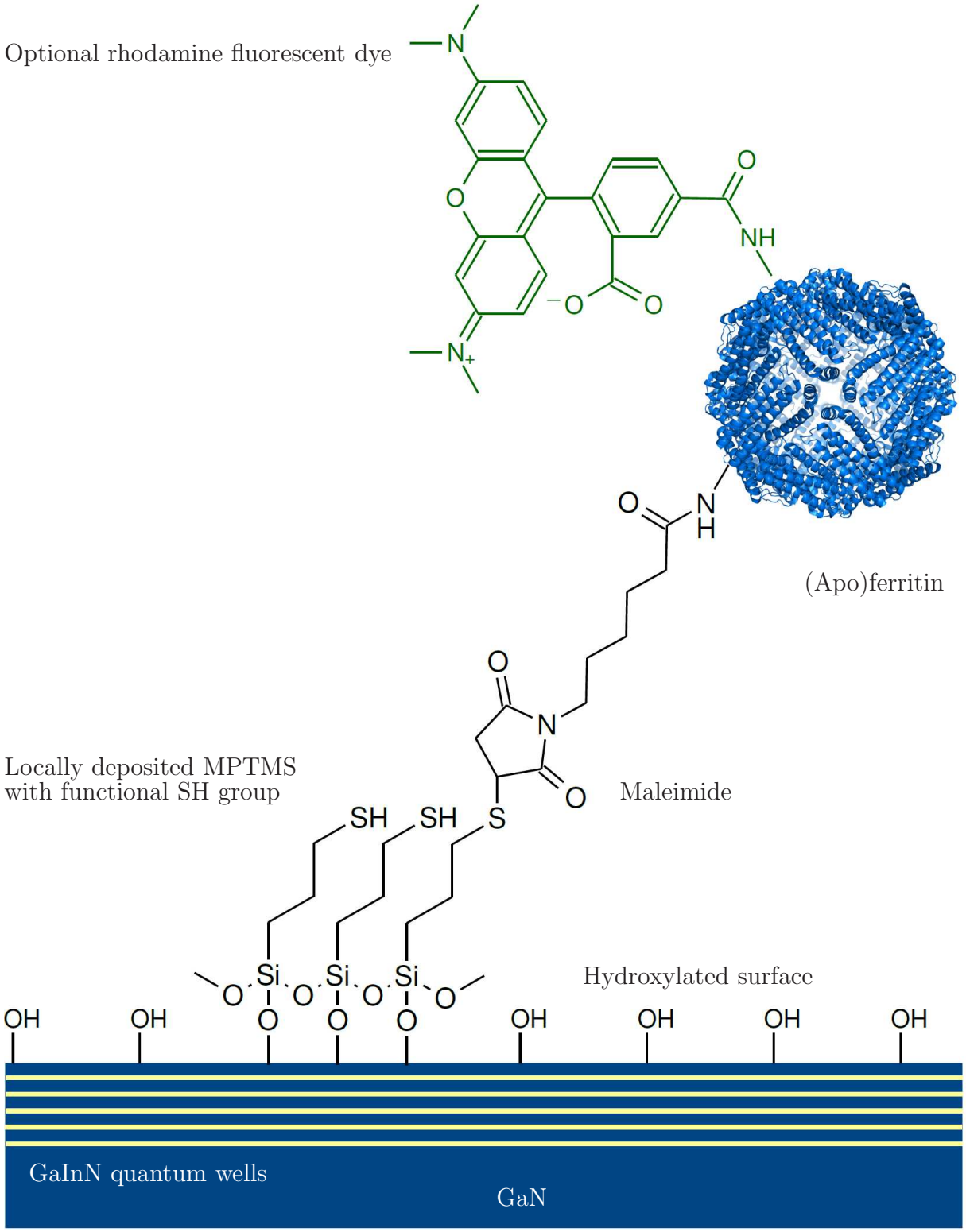

Fig. 1: Schematic illustration of the key-lock principle. Silane molecules are locally deposited onto the hydroxylated GaN surface. The functional SH group of MPTMS selectively binds to the maleimide group which is linked to the (apo)ferritin molecule in a separate functionalization step. Optionally, a fluorescent rhodamine dye can be attached to the (apo)ferritin molecules. Apoferritin was visualized with PyMol (PDB ID: 4V1W) [15].

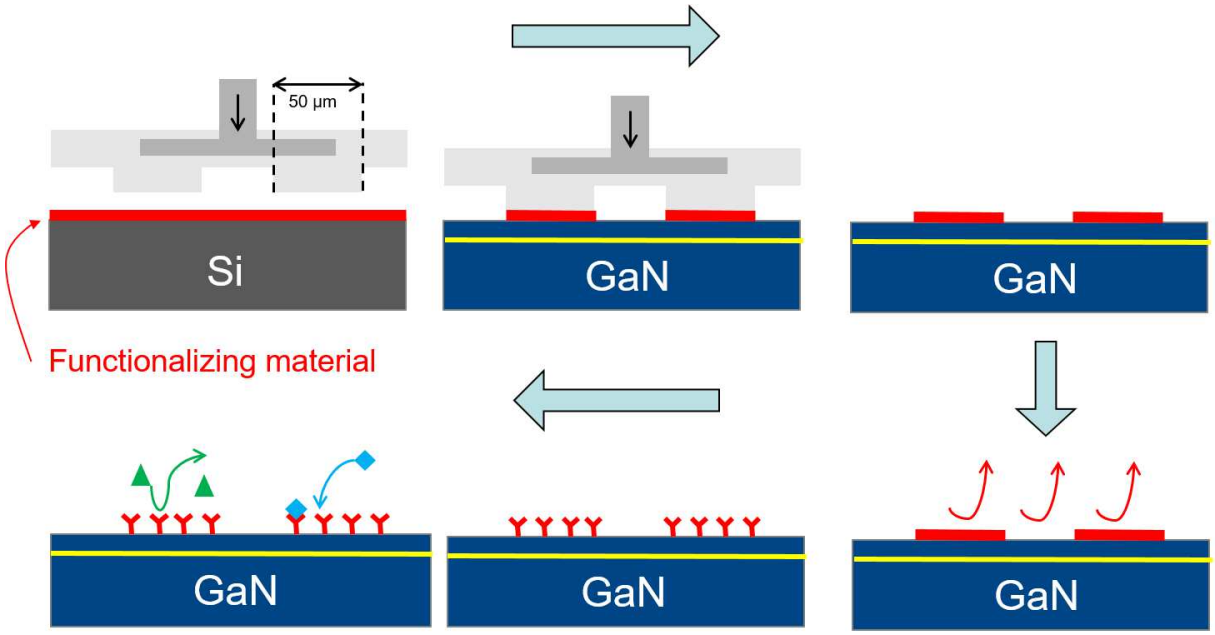

Fig. 2: Schematic illustration of the stamping procedure for selective deposition of silane on the hydroxylated GaN surface. In the first step, the PDMS stamp is loaded with MPTMS. Subsequently, the silane molecules are locally deposited on GaN and link to OH groups on the GaN surface. Unbound silanes are removed by rinsing the sample with toluene. The silanized areas on the surface allow a selective binding to the maleimide group of functionalized (apo)ferritin molecules.

3. Selective Binding of Ferritins

In our first experiments, a selective attachment of ferritin molecules on cheap glass substrates was studied in order to optimize the micro-contact patterning process. After silanization of the surface, (apo)ferritin molecules with functional maleimide group are attached to the SH group of the silane molecules. Ferritin molecules are drop-casted on the silanized surface and incubated for one hour. After incubation the samples are dipped in water in order to remove unspecifically bound ferritin molecules from the surface.

Fluorescent micrographs of selectively attached ferritin molecules incubated with high and low concentration are given in Fig. 3 (left, right), respectively. A selective attachment of fluorescent ferritin molecules on glass is clearly visible using fluorescence microscopy (Fig. 3). However, ferritin molecules also unspecifically attach in nominally not functionalized areas on glass. A periodic pattern with continuously increasing periodicity was chosen. Higher concentrations of ferritin are found in unpatterned areas with smaller periodicity of the stamping pattern. In areas with small periodicity silane molecules are expected to accumulate during the stamping process. Hence, fluorescent ferritins also attach in these areas.

Using the established micro-contact processing (Fig. 2), silane molecules are locally deposited on GaN surfaces. Due to the higher electrical conductivity of GaN in contrast to glass, scanning electron microscopy can be performed on the silanized GaN surface. A scanning electron micrograph of a silanized GaN surface is given in Fig. 4. Due to the

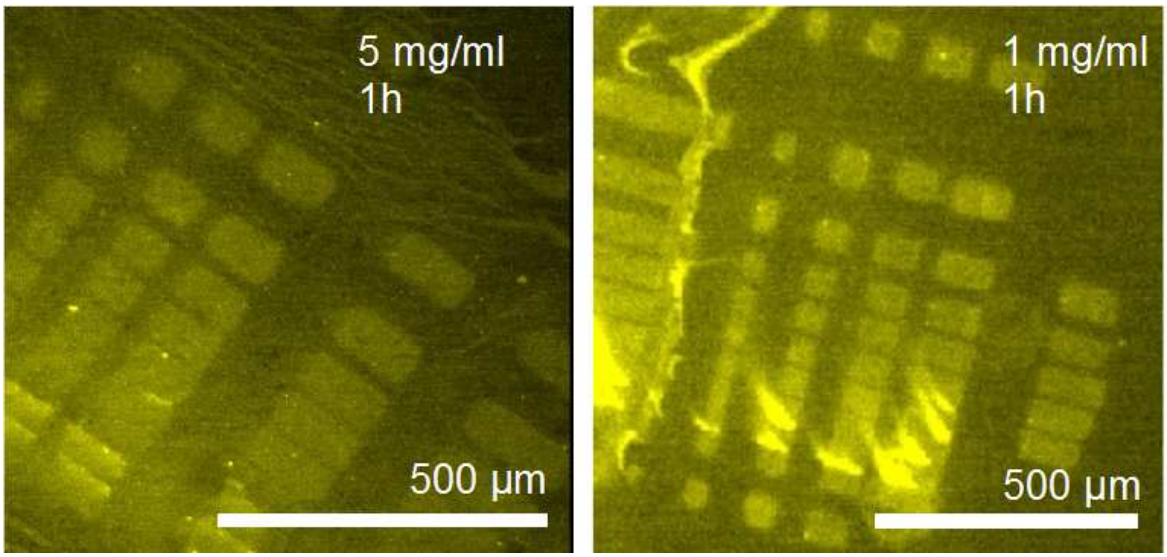

Fig. 3: Fluorescence micrograph of locally functionalized glass substrates using the silane stamping procedure after subsequent attachment of ferritin molecules. A selective attachment of fluorescent ferritin molecules is observed besides unspecifically bound ferritin in nominally not silanized areas.

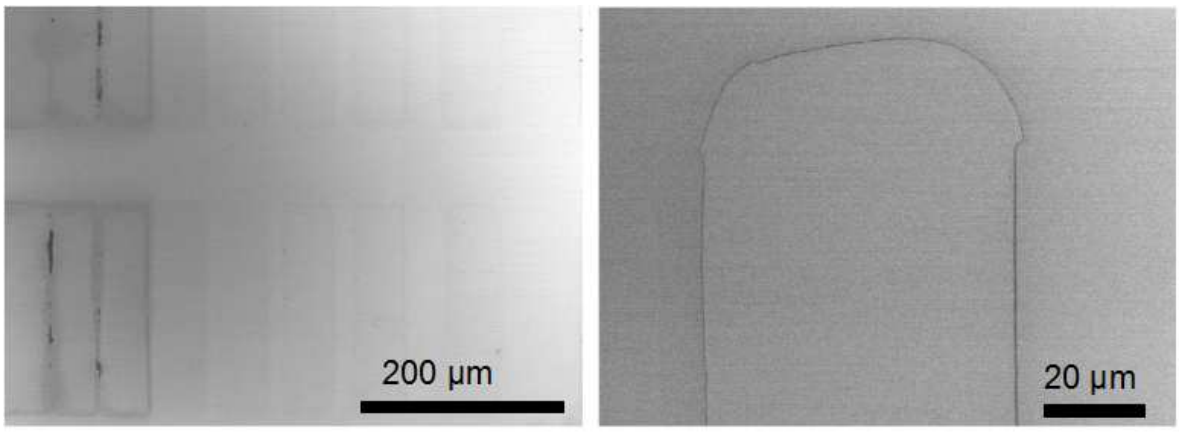

Fig. 4: Scanning electron micrograph of a silanized GaN surface using micro-contact patterning. Accumulations of MPTMS molecules are visible in areas with smaller periodicity of the stamp pattern (black areas).

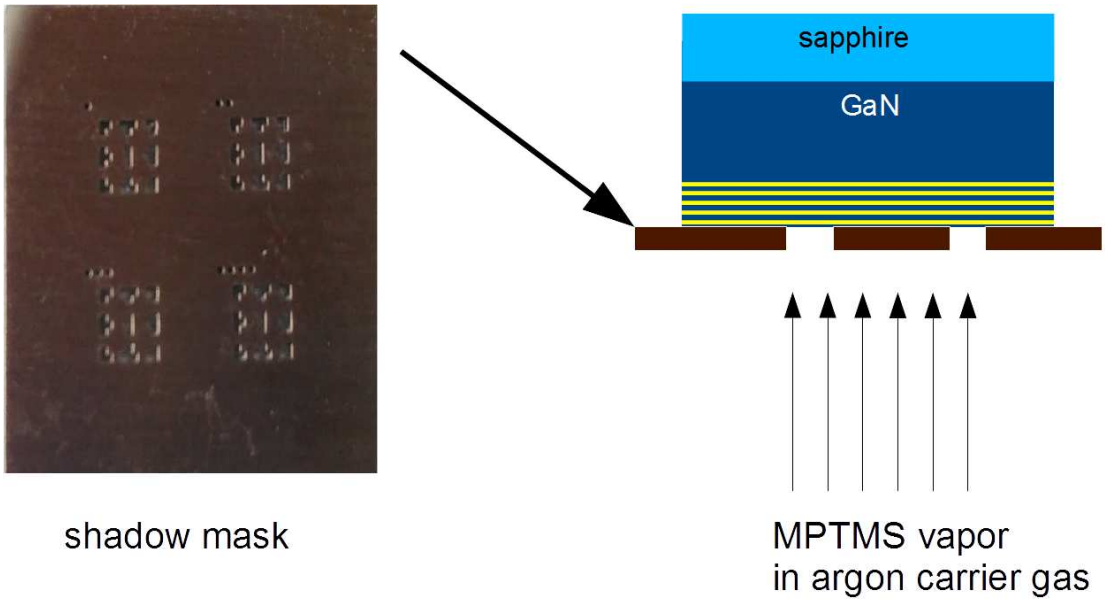

Fig. 5: Selective evaporation of silane molecules onto a masked GaN template.

material contrast (back-scattering) between the silanized and unpatterned GaN surface, the stamping pattern is clearly visible. As expected from our observations on glass substrates, accumulations of silane molecules are found in areas with small periodicity of the PDMS stamp pattern. Similar to our observations on glass, ferritin molecules are found to unspecifically bind in unpatterned areas (not shown).

In order to avoid contamination of nominally unpatterned areas with silane molecules during the micro-contact patterning, local evaporation of MPTMS onto a masked GaN surface is studied (Fig. 5). After silanization, a small droplet with ferritin is deposited on the patterned area and incubated for 1 hour. Subsequently, the sample is rinsed with ultrapure water to remove unspecifically bound ferritin.

In fluorescence and scanning electron microcopy, the shape of the original ferritin droplet is clearly visible (Fig. 6, left and right, respectively). A clear correlation of the attached ferritin molecules to the silane pattern is possible. However, again unspecifically bound ferritin molecules are found to preferably attach to unpatterned areas on GaN. Hence, GaN is found to be highly attractive for ferritin molecules and further investigations are necessary to remove unspecifically bound ferritin.

4. Summary

In this work, chemical functionalization of planar GaInN quantum well structures with silane molecules for local immobilization of ferritin molecules is demonstrated. Our studies provide a first step for next-generation biosensors using local photoluminescence changes of GaInN quantum wells interacting with ferritin molecules immobilized on the semiconductor surface. Two different approaches are studied based on micro-contact patterning and selective evaporation of silane molecules. A selective attachment of ferritin molecules on GaN is found as well as unspecifically bound ferritin on GaN.

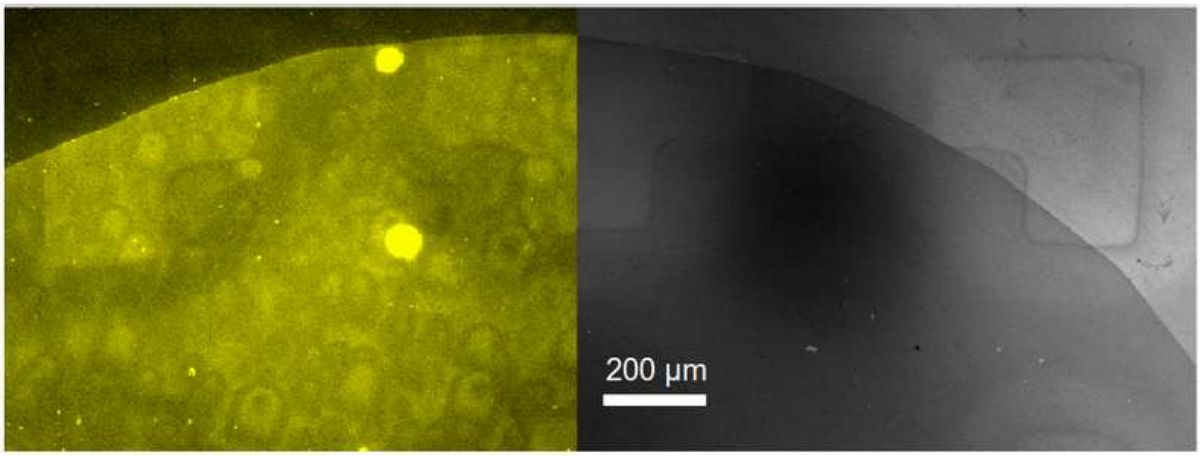

Fig. 6: Fluorescence micrograph of locally functionalized GaN templates using selective evaporation of MPTMS after subsequent local attachment of ferritin molecules (left), corresponding scanning electron micrograph (right). A selective attachment of fluorescent ferritin molecules can be observed besides unspecifically bound ferritin.

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References

[1] J. Teubert, P. Becker, F. Furtmayr, and M. Eickhoff, "GaN nanodiscs embedded in nanowires as optochemical transducers", *Nanotechnology*, vol. 22, pp. 275505-1–5, 2011.

[2] S. Paul, A. Helwig, G. Müller, F. Furtmayr, J. Teubert, and M. Eickhoff, "Optochemical sensor system for the detection of H_2 and hydrocarbons based on InGaN/GaN nanowires", *Sens. Actuators B*, vol. 173, pp. 120–126, 2012.

[3] K. Maier, A. Helwig, G. Müller, P. Becker, P. Hille, J. Schörmann, J. Teubert, and M. Eickhoff, "Detection of oxidising gases using an optochemical sensor system based on GaN/InGaN nanowires", *Sens. Actuators B*, vol. 197, pp. 87–94, 2014.

[4] B. Chu, C. Chang, S.J. Pearton, J. Lin, and F. Ren, "Recent advances in wide-bandgap semiconductor biological and gas sensors", Ch. 2 in *Semiconductor Device-Based Sensors for Gas, Chemical, and Biomedical Applications*, F. Ren and S. Pearton (Eds.), p. 43, Boca Raton: CRC Press, 2011.

[5] I. Cimalla, M. Gebinoga, A. Schober, V. Polyakov, V. Lebedev, and V. Cimalla, "AlGaN/GaN sensors for direct monitoring of nerve cell response", Ch. 1 in *Semiconductor Device-Based Sensors for Gas, Chemical, and Biomedical Applications*, F. Ren and S.J. Pearton (Eds.), p. 1, Boca Raton: CRC Press, 2011.

[6] K.A. Son, A. Liao, G. Lung, M. Gallegos, T. Hatake, R.D. Harris, L.Z. Scheick, and W.D. Smythe, "GaN-based high-temperature and radiation-hard electronics for harsh environments", in *Micro- and Nano-Harsh Environment Sensors*, T. George, M.S. Islam, and A.K. Dutta (Eds.), Proc. SPIE 7679, pp. 76790U-1–8, 2010.

[7] J. Eid *et al.*, "Real-time DNA sequencing from single polymerase molecules", *Science*, vol. 323, pp. 133–138, 2009.

[8] M.C. Estevez, M.A. Otte, B. Sepulveda, and L.M. Lechuga, "Trends and challenges of refractometric nanoplasmonic biosensors: a review", *Anal. Chim. Acta*, vol. 806, pp. 55–73, 2014.

[9] J.R. Heath and M.E. Davis, "Nanotechnology and cancer", *Annu. Rev. Med.*, vol. 59, pp. 251–265, 2008.

[10] A. Ermakova, G. Pramanik, J.M. Cai, G. Algara-Siller, U. Kaiser, T. Weil, Y.K. Tzeng, H.C. Chang, L.P. McGuinness, M.B. Plenio, B. Naydenov, and F. Jelezko, "Detection of a few metallo-protein molecules using color centers in nanodiamonds", *Nano Lett.*, vol. 13, pp. 3305–3309, 2013.

[11] M.J. Levene, J. Korlach, S.W. Turner, M. Foquet, H.G. Craighead, and W.W. Webb, "Zero-mode waveguides for single-molecule analysis at high concentrations", *Science*, vol. 299, pp. 682–686, 2003.

[12] Y. Ren and T. Walczyk, "Quantification of ferritin bound iron in human serum using species-specific isotope dilution mass spectrometry", *Metallomics*, vol. 6, pp. 1709–1717, 2014.

[13] V. Herbert, E. Jayatilleke, S. Shaw, A.S. Rosman, P. Giardina, R.W. Grady, B. Bowman, and E.W. Gunter, "Serum ferritin iron, a new test, measures human body iron stores unconfounded by inflammation", *Stem Cell*, vol. 15, pp. 291–296, 1997.

[14] D. Heinz, F. Huber, M. Spiess, M. Asad, L. Wu, O. Rettig, D. Wu, B. Neuschl, S. Bauer, Y. Wu, S. Chakrabortty, N. Hibst, S. Strehle, T. Weil, K. Thonke, and F. Scholz, "GaInN quantum wells as optochemical transducers for chemical sensors and biosensors", *IEEE J. Sel. Top. Quantum Electron.*, vol. 23, pp. 1900109-1–9, 2017.

[15] Visualization of PDB ID: 4V1W, C. Russo, and L. Passmore, "Ultrastable gold substrates for electron cryomicroscopy", *Science*, vol. 346, pp. 463–468, 2014, visualized with The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC.

[16] M. Brinkley, "A brief survey of methods for preparing protein conjugates with dyes, haptens and crosslinking reagents", *Bioconjugate Chem.*, vol. 3, pp. 2–13, 1992.