GaInN Quantum Wells as Optoelectronic Transducers for Biosensing of Ferritins

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In this work, investigations on gallium indium nitride (GaInN) quantum well structures as optochemical transducers in biosensing are presented. In contrast to the conventional electrical read-out of III-nitride-based sensors, a purely optical photoluminescence read-out is performed. Particularly, optical investigations of the iron-storage molecule ferritin deposited on GaInN quantum wells are presented. A significant spectral shift of the quantum well photoluminescence is observed for varying iron-load of ferritin.

1. Introduction

Currently, (bio)chemical sensing methods are often based on optical technologies [1–7]. Fluorescent labels which can be selectively linked by antibodies to different biomolecules [5] or even cells [8] are frequently applied. Most prominently, fluorescent markers are used in enzyme-linked immunosorbent assays (ELISA) which enables analytics down to the picomolar range [3] or in single molecule real time (SMRT) sequencing using fluorescent nucleotides [4, 5]. Today, the throughput in molecular analytics is often limited by the very weak light intensity of such fluorophors [3–5]. In particular, the signal-to-noise ratio is a crucial quantity for single molecule analytics [4, 5]. Fluorescent labels often suffer from photobleaching effects which limits the signal-to-noise ratio significantly [3, 9] or antibodies are not sensitive to specific molecular properties [10, 11].

As unbound iron is toxic, the iron-storage molecule ferritin plays an important role in the regulation of the iron concentration inside the human body [10]. The globular protein ferritin reversibly stores up to 4500 iron atoms inside its cavity where the ions undergo a mineralization process [10]. The concentration of ferritin molecules in human blood is typically determined using immunoassays. It is frequently monitored as biomarker in order to determine the iron status of the body [9, 10]. Ferritin bound iron is reported as a superior biomarker compared to ferritin protein levels [11]. Particularly, iron overload can be distinguished from an inflammation which also influences ferritin protein levels [11]. Additionally, other diseases such as hepatitis or cancer can influence the ferritin protein concentration [11]. A malfunction of the iron-incorporation and storage of the ferritin molecule is associated with Alzheimer's disease where higher concentrations of ferritin bound aluminum are found [12]. However, common antibodies used in immunoassays are only sensitive to the ferritin protein concentration but not to the iron load of the molecule itself [10, 11]. In contrast to immunoassays, determination of ferritin bound iron requires sophisticated methods such as species-specific isotope dilution mass spectrometry [10].

In recent years, gallium nitride (GaN) established as an excellent material system for general lighting and lasing applications in the visible spectral range. Besides classical applications in optoelectronics, group III-nitrides find increasing interest in (bio)chemical sensing [13–17]. Particularly, nanostructures such as nanowires are in the focus of research [13, 18–22]. Typically, nanostructures require rather sophisticated lithographic and epitaxial methods for their realization. Many studies involve an electrical read-out of nanostructures in (bio)chemical sensing [20–22]. However, group III-nitrides are expected to be particularly suited for optical sensing applications which so far has been rarely studied [13,18,19]. In contrast to other material systems, group III-nitrides benefit from an excellent chemical stability [23], biocompability [16, 17], optoelectronic properties [13, 18, 19], and polarity dependent piezoelectric properties [24, 25] which might be particularly useful in (bio)chemical sensing. In fact, the highest occupied and lowest unoccupied orbitals of many biomolecules match very well to the large bandgap of group III-nitrides [26].

In this work, an alternative "label-free" approach for molecular analytics without application of fluorophors is demonstrated using the stable photoluminescence (PL) of GaInN quantum wells. Particularly, the application of planar GaInN quantum well structures as optoelectronic transducers for sensing ferritin bound iron is investigated (Fig. 1). Planar, near-surface GaInN quantum wells allow a very flexible and reproducible arrangement of the optoelectronic properties compared to more sophisticated nanostructures. All investigations are based on the spectral and intensity changes of the GaInN quantum well luminescence in presence of ferritin with varying iron-load.

2. Experimental

GaInN quantum well structures are grown in a commercial Aixtron AIX200/RF metal organic vapor phase reactor using ammonia (NH_3), trimethylgallium (TMGa), trimethylaluminum (TMAl), triethylgallium (TEGa), and trimethylindium (TMIn) as precursors. Ultra-pure hydrogen and nitrogen are applied as carrier gases.

First, a thin oxygen-doped aluminium nitride (AlN) nucleation layer is grown on *c*-oriented sapphire wafers with a miscut of 0.3° towards the *a*-direction. Subsequently, a Ga-polar GaN buffer layer with a thickness of about 1 µm is realized followed by five GaInN multi quantum wells with a nominal thickness of 3 nm and 7 nm GaN barrier. In order to enable a sensitivity of the quantum wells to the biomolecules at the surface, a thin GaN cap layer with a thickness of about 7 nm is realized.

Before deposition, the GaInN quantum well surface is hydroxilated using a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) with a ratio of 1:1 for 5 min. Subsequently, the samples are rinsed in deionized water and dryed under nitrogen flow. Ferritin and apoferritin (corresponds to ferritin without iron-load) from horse spleen are purchased from Sigma-Aldrich und purified using dialysis. In particular, the saline and glycol buffer solutions of the biomolecules are substituted by deionized water. A concentration of about 2 µmol dm^{-3} of (apo)ferritin in deionized water is realized and deposited on the hydroxilated GaInN quantum well surface.

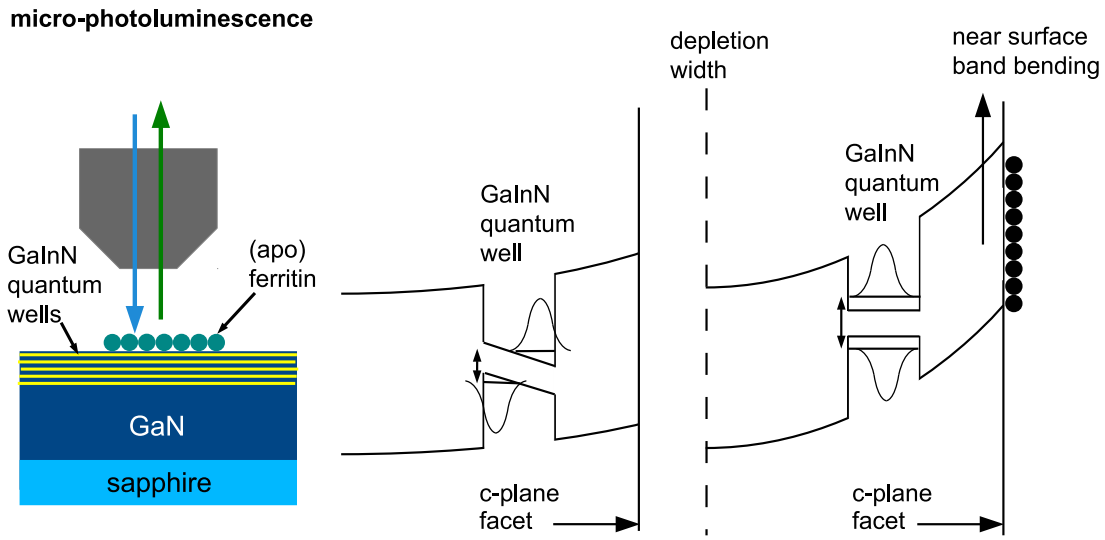

Fig. 1: Schematic illustration of the micro-photoluminescence setup for front side excitation of modified GaInN quantum well structures (left). Near-surface band structure of a polar single GaInN quantum well surface without biomolecule modification (middle) corresponding band structure with increasing band bending after adsorption of oxidizing molecules (right).

Photoluminescence measurements are performed using a micro-photoluminescence setup with mirror objective (Fig. 1). A HeCd laser with main emission wavelength at 325 nm is applied for excitation of the quantum well photoluminescence. The optical read-out is performed using a monochromator and a liquid nitrogen-cooled CCD camera.

Local backside excitation PL experiments are performed using backside polished GaInN quantum well structures. GaInN quantum well samples are mounted on a pinhole in order to allow local measurements before and after deposition of the biomolecules at the same position on the sample surface. A blue laser diode with an emission wavelength at 405 nm is used for resonant excitation of the quantum wells in order to avoid absorption inside the GaN buffer layer. The PL read-out is performed from the front side.

3. Influence of Ferritin Bound Iron on the GaInN Quantum Well Photoluminescence

Ferritin and apoferritin are locally deposited on a hydroxilated planar structure containing 5 GaInN quantum wells. Photoluminescence spectra measured from the front side are shown in Fig. 2.

A significant spectral shift and an intensity variation of the local photoluminescence response is observed after deposition of the biomolecules on the surface. In order to exclude the influence of local fluctuations of the quantum well photoluminescence, several local measurements are performed on ferritin and apoferritin areas.

A mean spectral red-shift of about 2.4 nm and an intensity increase of about 35 % is observed for the areas where apoferritin is deposited with respect to ferritin. Regarding the Ga-polarity and *c*-orientation of the GaInN quantum wells, a red-shift for apoferritin

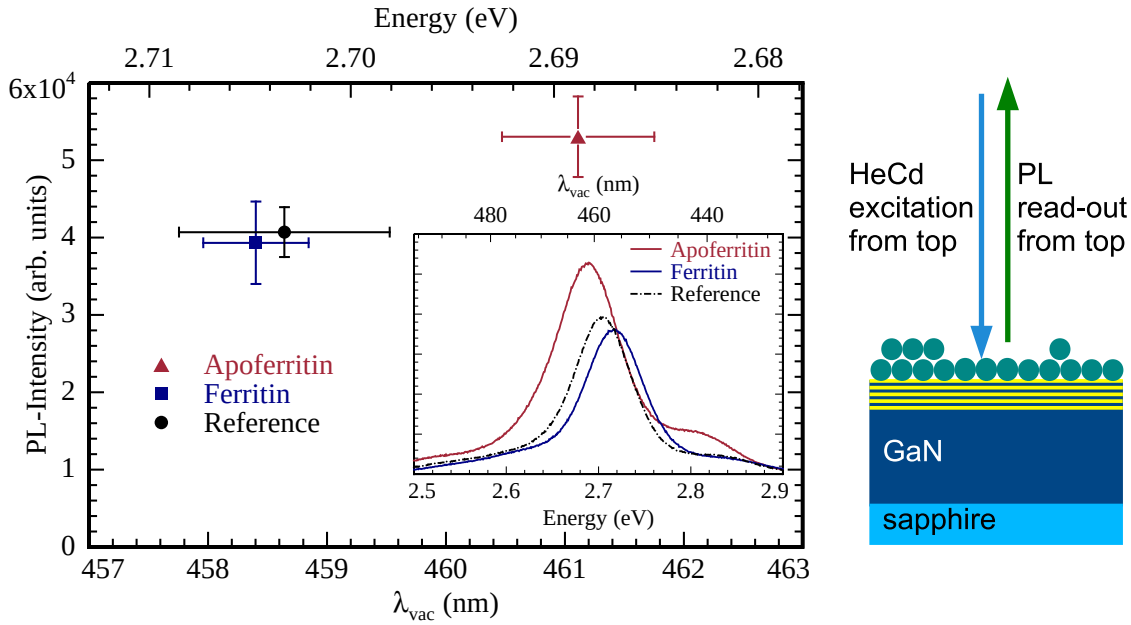

Fig. 2: Micro-photoluminescence of planar 5× GaInN multi quantum wells with ferritin and apoferritin surface modification and corresponding reference structures without modification (left). Error bars are mainly caused by local fluctuations of the quantum well PL and local inhomogeneity of the deposited biofilm thickness (right).

corresponds to a reduction of the initially upward surface band bending. A reduced near-surface band bending leads to an increased tilting of the quantum well band structure and hence to an increased quantum confined Stark effect (QCSE). Therefore, apoferritin can be assumed to behave as a reducing agent which attracts holes at the GaN surface and hence reduces the band bending.

Indeed, it is reported in literature that apoferritin can bind different kinds of positively charged metal ions, particularly Fe^{2+} and Fe^{3+} ions [27]. The binding affinity is reported to be pH-dependent as protons in the buffer solutions compete with other metal ions [27, 28]. Ferritins are reported to obey a negative surface potential which attracts cations [29]. Surprisingly, the photoluminescence intensity slightly increased after apoferritin deposition. A reduced overlap of the electron and hole wave functions and reduced radiative recombination propability is expected due to the increased QCSE. The intensity increase might be explained by an improved light extraction due to the increased surface roughness or by reduced non-radiative surface recombination.

In contrast to apoferritin, a tendency for a blue shift with respect to the reference structure without surface modification is observed for ferritin. It is expected that the reducing nature of apoferritin is significantly weakened or overcompensated due to the incorporation of positively charged ions, which is indeed observed. The iron concentration inside the ferritin molecules is expected to vary. Unfortunately, local fluctuations of the quantum well PL and large error bars inhibit a clear statement about the electron transfer for ferritin. A spectral blue shift corresponds to an oxidizing nature of ferritin. This corresponds to an increase of the near-surface band bending and reduced QCSE.

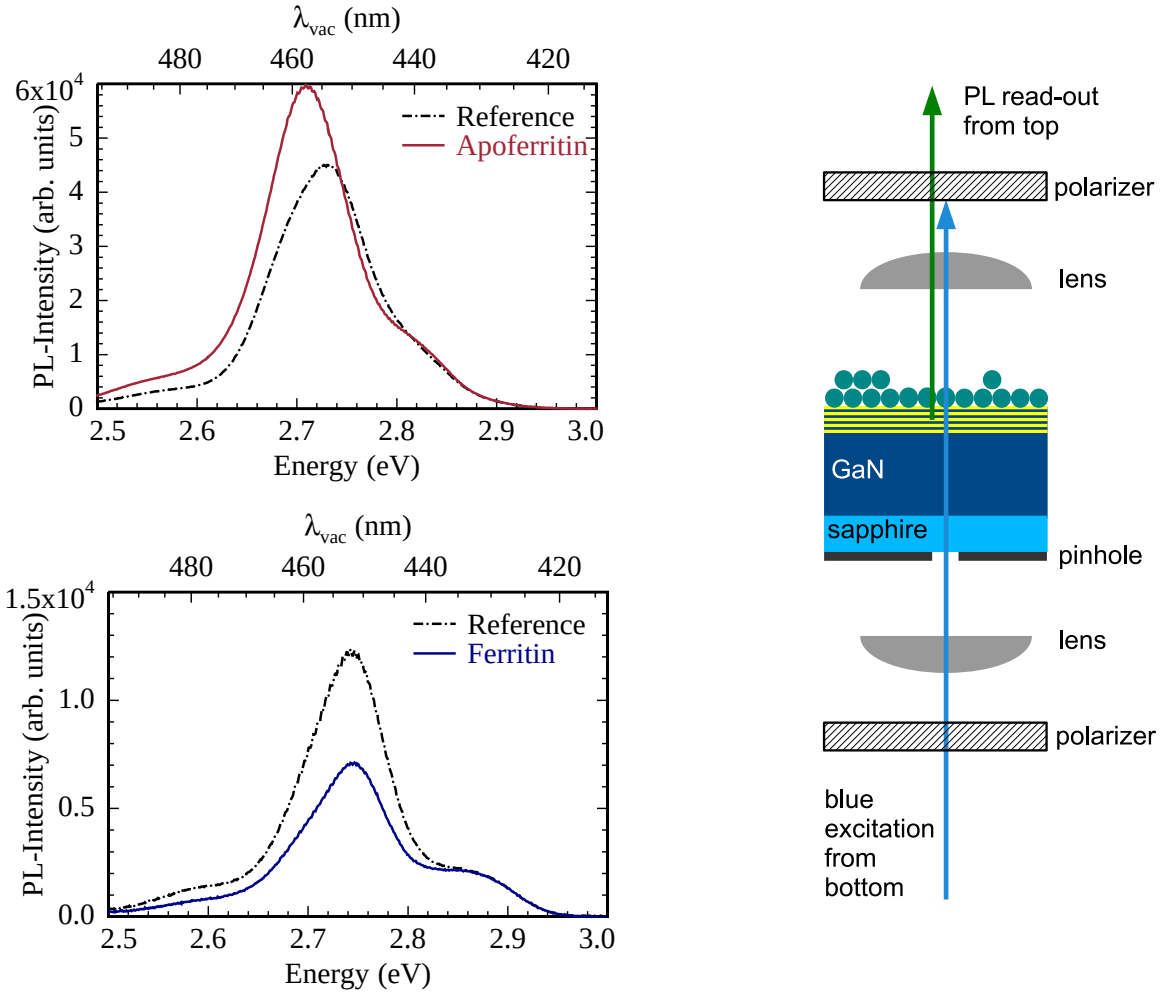

Fig. 3: Local backside excitation PL measurement using double polished 5× GaInN multi quantum well structures (left) for apoferritin (top) and ferritin (bottom) modified surfaces. Schematic illustration of the setup (right): a polarized blue 405 nm laser is used for resonant excitation of the quantum wells which is selectively separated from the PL using a second polarizer. The sample is mounted on a pinhole to excite the same position before and after deposition of the biomolecules.

In order to exclude the influence of local variations of the quantum well PL, local backside excitation experiments are performed. The polished sapphire backside of a planar multi-quantum well structure is mounted on a pinhole (Fig. 3). As GaN is absorbing in the UV spectral range, a blue laser diode is used for optical resonant excitation of the GaInN quantum well PL. The optical PL read-out is again performed on the quantum well side.

Again a spectral redshift is found after deposition of apoferritin biomolecules on the quantum well surface compared to the local reference measurement without surface modification. A slightly higher intensity is found which might be again explained by improved PL light extraction on the quantum well surface. The result is in very good agreement with the previously described investigations on the front side. In contrast, a reduced spectral shift with a slight tendency for smaller wavelength is found for ferritin molecules

which again matches with our expectations. However, the PL quenching is much more pronounced compared to our frontside excitation experiments which might be a local effect. Obviously, the overall iron load of (apo)ferritin molecules seems to have a strong influence on the optoelectronic properties of polar GaInN quantum wells. A reduced spectral shift of ferritin molecules might be explained by a reduced binding affinity of ferritin for positive charges compared to apoferritin.

4. Summary

The realization and application of planar GaInN quantum well structures as optical transducer elements in biosensing is demonstrated. Ferritin molecules on the surface of such structures strongly influence the near-surface quantum well PL depending on the iron-load of the molecules. A significant spectral redshift of the photoluminescence is observed in the presence of apoferritin caused by the externally induced QCSE. The results might be particularly useful for new "label-free" optical sensor concepts without application of fluorophors based on the transducing properties of GaInN quantum wells.

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