静岡大学　博士論文

バイオイメージング応用のためのプラズマ処理酸化亜鉛ナノ材料に関する研究

ミハイ アレクサンドル チオラン

静岡大学
大学院自然科学系教育部
ナノビジョン工学専攻

2014 年 6 月

Doctoral Thesis

Study of zinc oxide nanomaterials for bioimaging applications using plasma treatment

Mihai Alexandru Ciolan

Graduate School of Science and Technology
Educational Division
Department of Nanovision Technology
Shizuoka University

June 2014

Table of contents

Abstract

This current study focuses on the functionalization of the zinc oxide to be able to put the bases for development of new bioimaging techniques that can detect some specific biological processes. We promote the use of zinc oxide due to his low toxicity compared with other semiconductor based materials and his optical properties, as photoluminescence, at room temperature.

The motivation for this study relies in two aspects: one is the use of new materials that can fulfill specific roles when interacting with the living organism, the second one is the method used to prepare the interface between the nanomaterials and the biological medium.

There is a lack of reports regarding the use of zinc oxide based materials for imaging report and thus we promote it due to its low toxicity compared with other semiconductor based materials and its optical properties, as photoluminescence at room temperature.

Till now, reports about the functionalization of zinc oxide by wet chemical methods were given but these methods have some major disadvantages when dealing with materials that should be compatible with living organism like: increased toxicity due to the chemicals used for functionalization, agglomeration of the nanoparticles, low dispersion in aqueous media and so on.

For the first time we promote the use of low temperature plasma as a functionalization method for zinc oxide trying to overcome some of the issues of the wet chemical methods.

Non-equilibrium low and atmospheric pressure plasma techniques have been recognized for a long time as efficient surface modification techniques, and accordingly the plasma approach was also considered for the generation of biocompatible surfaces. Plasma-surface modification is an effective and economical surface treatment technique for many materials and of growing interests in biomedical engineering. The unique advantage of plasma processing is that the surface properties and biocompatibility can be selectively enhanced, thus enhancing the performance of the nanomaterials.

I conducted my study by first investigating the possibility of adding amine groups on the surface of zinc oxide commercial powder by employing ammonia excited surface wave plasma as a processing method. Plasma parameters were analyzed by optical emission spectroscopy for better understanding of how the functionalization can be achieved. The introduction of amine groups was analyzed by XPS measurements and also confirmed employing chemical derivatization.

The next step of the research consisted in the functionalization of zinc oxide nanoparticles synthesized by pulsed laser ablation. Also new condition for the plasma were analyzed for optimization of the process. Ar feed into the discharge proved to enhance the introduction of amine groups on the surface of the zinc oxide and thus better performance for the processing was achieved. Plasma was investigated furthermore by optical emission spectroscopy and high-performance plasma sampling mass spectrometry for different ratios of Ar and NH_3 used in the mixture to excite plasma.

To fulfill our initial goal we checked how the photoluminescence of the zinc oxide changes depending on the plasma processing of the samples. Quantification of the number of amine groups was also performed by chemical derivatization. Finally, we used the amine functionalities on the surface of the zinc oxide as reactive sites to connect biomolecules.

Introduction

1.1 Background

To visualize and understand the cellular and molecular biological processes, it is mandatory to make use of materials that poses the same size as the biomolecular components of interest. Recent advances in nanomaterials and nanotechnology provide such tools, as quantum dots for example, used to obtain essential information about biological processes[1]–[4]. Disease treatment is reaching a turning point, with the emerging fields of molecular based medicine and personalized medicine. A cellular level control is not possible without nanotechnology to provide high sensitivity and specificity. The possibility of developing multifunctional nano-devices is attracting more attention.

For biomedical applications such as imaging and drug delivery, size is one of the most important aspects for success because macro size materials have notable drawbacks (e.g. inadequate targeting and toxicity) compared with nanosize with respect to the cellular interactions. To understand the processes that stand at the basis of the biological processes, the use of nanomaterials is desirable; in the same time only downscaling the size of the material it is not enough. Further preparation of the nanoparticles is most of the time necessary, by giving them specific functions. This special preparation usually involves preparation of the nanomaterials surface as to be compatible to the biological media process called functionalization. Functional groups or molecules with functional ends are grafted on the surface creating an interface suitable for the desired aim.

Bioimaging techniques offer the possibility of characterization and visualization of biological processes at nanometer scale giving precise and real time information. Due to the fact that most biological processes take place at nanometer scale, the use of nanomaterials with specific function is of great interest for ideal imaging resolution. By adding functional groups to the surface of nanoparticles (NPs), the sensitivity and selectivity for various biospecies can be improved and new technologies can be developed.

Disease treatment is reaching a turning point, with the emerging fields of molecular based medicine and personalized medicine. A cellular level control is not possible without nanotechnology to provide high sensitivity and specificity. The possibility of developing multifunctional nano-devices is attracting more attention.

Biocompatibility is a necessary property of materials during their interaction with biological systems; scientists and engineers who are dealing with medical and biological problems extensively investigate this aspect. It is a complex phenomenon and its mechanisms are not fully understood, mainly due to the lack of detailed knowledge of *in vivo* conditions. The degree of biocompatibility sets the limits to engineering solutions required for biomedical problems. Conventional wet-chemistry approaches employed for changing the physical–chemical characteristics of material surfaces often have application limitations owing to the complexity of the techniques, the requirement of environmentally non-friendly processes, and labor-intensive and expensive methodologies.

Non-equilibrium low and atmospheric pressure plasma techniques have been recognized for a long time as efficient surface modification techniques, and accordingly the plasma approach was also considered for the generation of biocompatible surfaces. Surface functionalization processes involving non-depositing plasma-gases, and plasma-enhanced deposition reactions have been investigated for the modification of biomaterial surfaces.

Plasma-surface modification is an effective and economical surface treatment technique for many materials and of growing interests in biomedical engineering. The unique advantage of plasma processing is that the surface properties and biocompatibility can be selectively enhanced, thus enhancing the performance of the nanomaterials.

1.2 Objectives of the research

The novelty of this study relies in two aspects: one is the use of new materials that can fulfill specific roles when interacting with the living organism, the second one is the method used to prepare the interface between the nanomaterials and the biological medium. For the first novel aspect, we focus on the functionalization of the zinc oxide for the development of bioimaging techniques. We promote the use of zinc oxide due to its low toxicity compared with other semiconductor based materials and its optical properties, as photoluminescence at room temperature.

Until now, reports about the functionalization of zinc oxide by wet chemical methods were given but this methods have some major disadvantages when dealing with materials that should be compatible with living organism like: increased toxicity due to the chemicals used for functionalization, agglomeration of the nanoparticles, low dispersion in aqueous media and so on.

The second novel point of this thesis is brought by the processing technique. To the best of our knowledge, we are the first to promote the use of low temperature plasma as a functionalization method for zinc oxide aiming to overcome some of the issues of the wet chemical methods. Microwave-excited surface wave plasma has been already proven suitable for the production and modification of several nanomaterials [5]–[8].

First of all we tested the possibility to functionalize the zinc oxide powder by plasma processing to check if this process works. Optical emission spectroscopy of the plasma was carried out and the influence of parameters like power and bias voltage were investigated.

Going further, we changed the micrometer size powder with zinc oxide nanostructures obtained by pulsed laser ablation deposition technique. These nanostructures are characterized by considerable smaller size, improved optical properties and higher surface to volume ratio compared with the bulk material.

Next step of the study was to optimize the introduction of the amine groups on the surface of zinc oxide by exciting the surface wave plasma in different mixtures of argon and ammonia. The optimization was correlated with detailed plasma diagnosis by high resolution plasma sampling mass measurements.

1.3 Synopsis of the chapters

This thesis consists of 7 chapters.

Chapter 1 represents the general introduction of the thesis with background and motivation.

Chapter 2 is an introduction to nanomaterials with emphasis on zinc oxide. In this chapter zinc oxide properties, applications, and methods of obtaining are briefly described.

Chapter 3 provides brief information regarding the functionalization of nanomaterials and current advances in bioimaging applications. Detailed information about zinc oxide functionalization obtained by wet chemical methods and possible application are also discussed. From this chapter the main motivation for the use of plasma processing as functionalization method arises.

Chapter 4 presents the methods employed during this study to analyze and characterize the outcome of the plasma processing in term of functionalization. Detailed about the fabrication of the zinc oxide nanomaterials by laser ablation and information about the surface wave plasma and analysis by different measurements are also discussed.

Chapter 5 includes original results regarding the functionalization of the zinc oxide materials and also about the surface wave plasma analysis by different measurements. These results play an important role for the understanding how plasma functionalization works and how can the process be optimized.

The possibility of adding functional groups on the surface of zinc oxide is correlated with the plasma parameters and is presented in detailed in *Chapter 6*. Optimization of the processing techniques is achieved by investigating in detail the plasma parameters by optical emission spectroscopy and high-performance plasma sampling mass spectrometry. Alongside results about the quantification and the photoluminescence of the functionalized zinc oxide are presented. The possibility of connecting biomolecules to the functionalities is also shown.

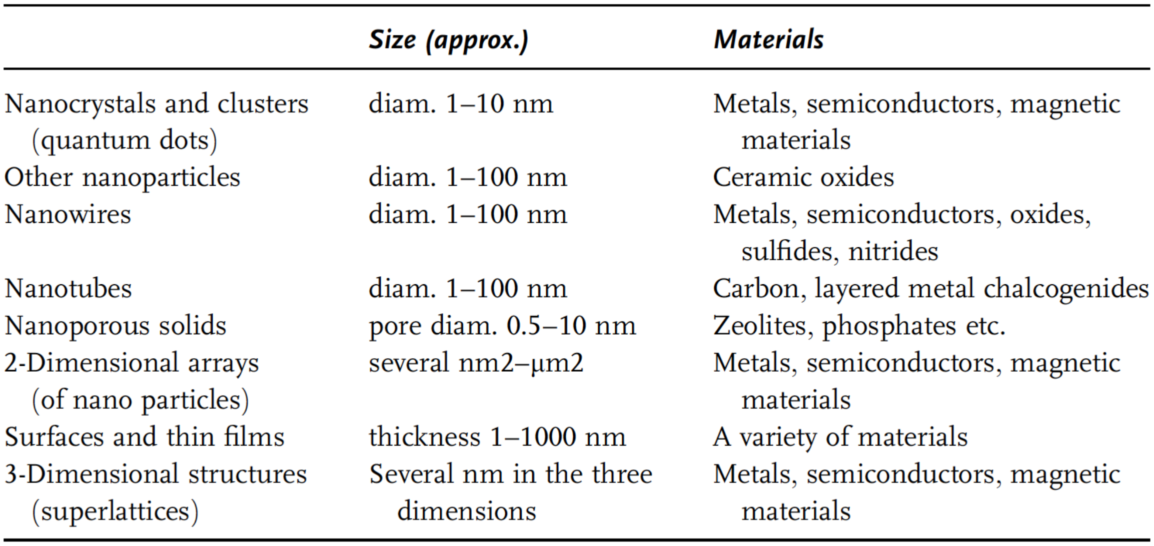
Chapter 7 summarizes the key points of the thesis.

Nanomaterials

The remarkable properties of the nanomaterials influence the modern technical society in many interesting aspects. Downscaling the size of the bulk material to reach the nanosize is very interesting from the point of view of the nanotechnology because the nanosystems do not behave the same as the bulk counterparts. The interest towards the nanoscale technology is due to the scientific ability to fabricate and manipulate things with a small number of atoms for the development of applications.

The properties of the nanomaterials depend on the extrinsic contribution (the surface phenomena) and the intrinsic contribution (quantum confinement effects). Another interesting point of view regarding the nanostructured materials is the surface to volume ratio, which rapidly increases when the particle size decreases. This particular property of the nanomaterials determines the extent activity of the nanoparticulate system.

Table 1. Examples of nanomaterials [1].



	Size (approx.)	*Materials*
Nanocrystals and clusters (quantum dots)	diam. 1–10 nm	Metals, semiconductors, magnetic materials
Other nanoparticles	diam. 1–100 nm	Ceramic oxides
Nanowires	diam. 1–100 nm	Metals, semiconductors, oxides, sulfides, nitrides
Nanotubes	diam. 1–100 nm	Carbon, layered metal chalcogenides
Nanoporous solids	pore diam. 0.5–10 nm	Zeolites, phosphates etc.
2-Dimensional arrays (of nano particles)	several nm2–μm2	Metals, semiconductors, magnetic materials
Surfaces and thin films	thickness 1–1000 nm	A variety of materials
3-Dimensional structures (superlattices)	Several nm in the three dimensions	Metals, semiconductors, magnetic materials

Nanosciece and nanotechnology primarily deal with the synthesis, characterization, manipulation and implementation of the nanostructured materials. Nanostructures can assemble in many ways: clusters, quantum dots, nanocrystals, nanowires, nanotubes, and, to a bigger extent, they can be arranged in specific arrays. Table 1 depicts some typical dimensions of the nanomaterials [9]

2.1 Zinc oxide based materials

Zinc oxide is a II-VI semiconductor material that attracted a big interest in the research community due to its interesting properties that promoted it as a suitable material for developing diverse applications. One of the most important is its large exciton binding energy (60 meV) that could lead to lasing action based on exciton recombination and possibly polariton/exciton interaction even above room temperature and with very low threshold currents. The history of zinc oxides research dates from many decades ago but only since the beginning of 20th century reports have been given on the lattice parameter [10], the Raman scattering studied vibrational properties [11], the first growing of epitaxial zinc oxide growing on sapphire by chemical vapor deposition [12]. Based on this knowledge zinc oxide materials applications emerged: Au Schottky barriers (1965), light-emitting diode (LED) (1967), and metal insulator semiconductors (MIS) with Cu_2O p-type material (1974), ZnO/ZnTe n-p junctions (1975).

2.2 Crystallographic structure

Semiconductors compounds from group II-VI usually crystallize in cubic, zinc blende or wurtzite (Wz) structure – each anion of the material is surrounded by four cations at the corners of a tetrahedron and vice versa. Being part of the II-VI compound group zinc oxide ionicity resides at the borderline between the covalent and ionic semiconductors.

The crystallographic structure of zinc oxide can be wurzite (B4), zinc blende (B3), and rocksalt (or Rochelle salt) as shown in Figure 1.

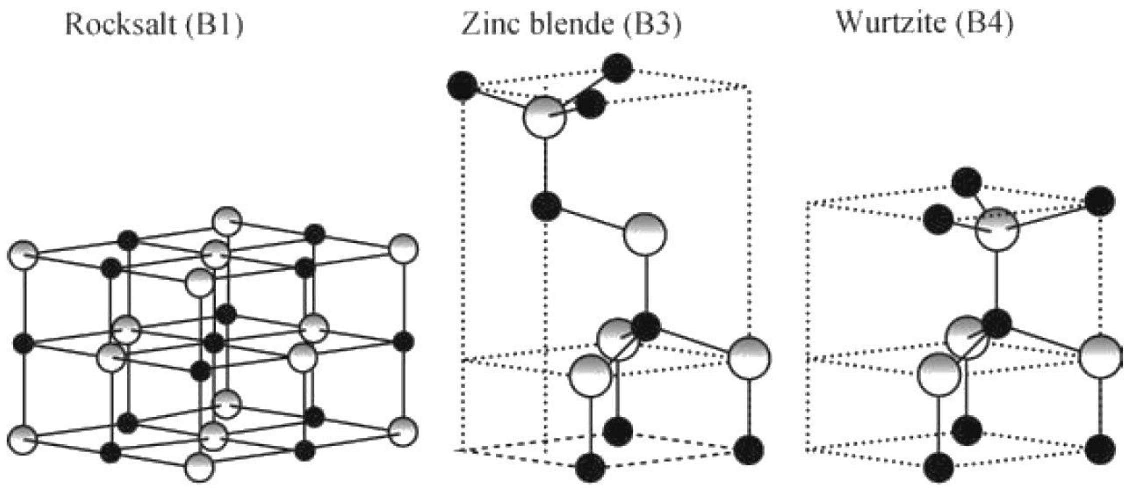


Figure 1. Representation of the ZnO crystal structures: cubic rocksalt (left), cubic zinc blende (center), and hexagonal wurtzite (rigth) [10]**.**

The wurtzite symmetry is one of the thermodynamically stable phases in normal ambient conditions. The other phase can be stabilized like the wurtzite symmetry but it needs specific high pressure and temperature conditions. The ideal wurtzite structure is characterized by a hexagonal unit cell with two lattice parameters, *a* and *c,* with a ratio of $\frac{c}{a} = \sqrt{\frac{8}{3}} = 1.633$, and two interpenetrating hexagonal closedpacked sublattices with one atom displaced with respect to the other along the threefold *c*-axis by the amount of *u=0.375*, which represents the length of the bond parallel to the *c*-axis (anion-cation bond length or the nearest-neighbor distance) divided by the *c* lattice parameter. Figure 2 shows a detailed schematic of the wurtzite structure. Changing the *c/a* ratio or the *u* value, the wurtzite structure of the zinc oxide deviates from the ideal model. The experimental values of the *c/a* ratio are smaller than the ideal and strongly correlate with the *u* parameter. When the ratio decreases, the long-range polar interactions cause a distortion of the tetrahedral angles and, thus the four tetrahedral distances remain constant while the *u* parameter increases.

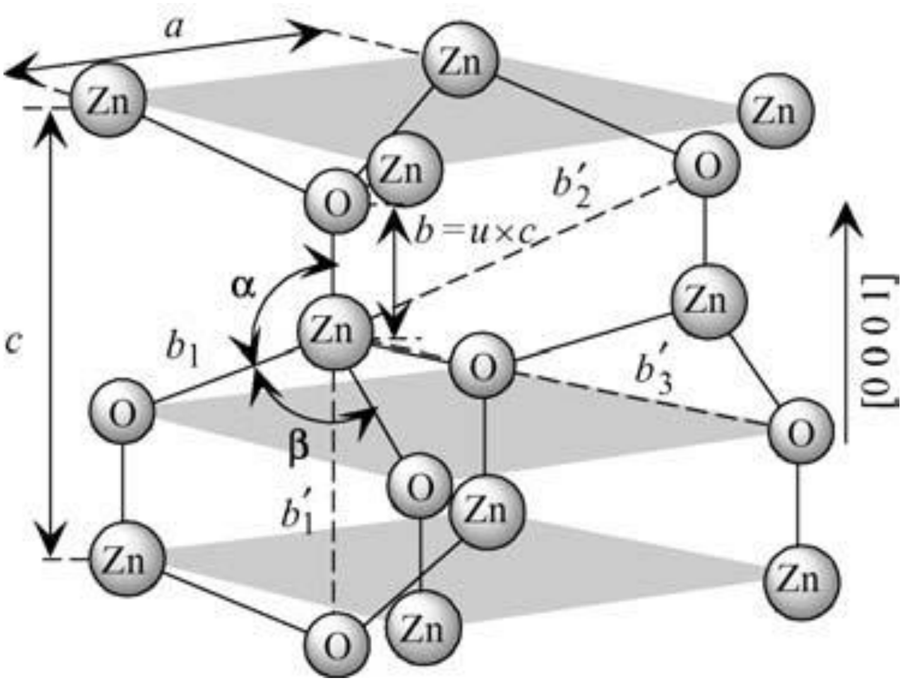


Figure 2. Wurtzite structure of zinc oxide with lattice constants [13]**.**

Zinc oxide can also crystalize in a blende structure that is metastable and can be stabilized only by heteroepitaxial growth on cubic substrates (as ZnS, GaAs/ZnS) reflecting the topological compatibility to overcome the tendency of forming wurtzite phase. Zinc oxide can be transformed in *rocksalt* structure using external hydrostatic pressure which reduces the lattice dimensions and causes the interionic Coulomb interactions favoring the ionicity over the covalent nature.

2.3 The lattice of zinc oxide

Lattice parameters of the zinc oxide were intensively studied, the experimental measurements at room temperature are in good correlation with the theoretical calculations for the ideal wurtzite structure as seen in Table 2. The slightly differences from the ideal wurtzite crystal could be attributed to lattice instability and ionicity.

Table 2. Measured and calculated lattice constants and the u parameter of wurtzite zinc oxide

Wurtzite				
a (Å)	*c (Å)*	*c/a*	*u*	*Reference*
		1.633	0.375	*Ideal*
3.2496	5.2042	1.6018	0.3819	[a]
3.2501	5.2071	1.6021	0.3817	[b]
3.286	5.241	1.595	0.383	[c]
3.2498	5.2066	1.6021		[d]
3.2475	5.2075	1.6035		[e]
3.2497	5.206	1.602		[f]
		1.593	0.3856	[g]
		1.600	0.383	[h]

[a] Measured using XRD [13].

[b] Measured using XRD [4].

[c] Calculated using ab initio periodic LCAO method, based mainly on the Hartree–Fock Hamiltonian, with an all-electron Gaussian-type basis set [32].

[d] Measured using EDXD [12].

[e] Measured using XRD [9].

[f] Measured using XRD [30].

[g] Calculated using first-principles periodic Hartree–Fock LCOAO program [20].

[h] Calculated using ab initio quantum mechanical level through the Berry phase scheme applied delocalized crystalline orbitals and through the definition of well-localized Wannier functions [33].

2.4 Zinc oxide properties and developed applications

2.4.1 Direct and wide band gap

The 3.44 eV band gap at low temperatures and 3.37 eV at room temperature [5,6][14][15] enable the possibility to use zinc oxide in a range of optoelectronics applications, mainly in the blue/UV region. These include light-emitting diodes, laser diodes, and photodetectors [16][17]. Optically pumped lasing was achieved in ZnO platelets [18] [19], thin films [18],various clusters of zinc oxide nanocrystals[20], and nanowires[21]. p-n homojunctions were also reported [22] [23] [24].

2.4.2 Exciton binding energy

Zinc oxide has a 60 meV free exciton binding energy [18] which is much larger compared with other semiconductor based materials (GaN) [25]. Thus, zinc oxide has an efficient excitionic emission at room temperature and above it [18], [19]. This property (based on the fact that the oscillator strength of excitons is typically larger than of direct electron-hole transitions in direct gap semiconductors [26]) makes zinc oxide a promising candidate for optical devices based on excitonic effects.

2.4.3 Piezoelectric constants

Piezoelectricity is the property of a material to get a deformation in the crystal lattice when a voltage is applied and vice versa. These kinds of materials were successfully implemented in sensors, transducers, and actuators. The wurtzite zinc oxide is characterized by a low symmetry combined with the large electromechanical coupling – zinc oxide materials exhibit strong piezoelectric and pyroelectric properties. Reports proved the possibility to obtain piezoelectric zinc oxide film with uniform thickness and orientation on a variety of substrates and with diverse deposition techniques such as: sol-gel process, molecular beam epitaxy, and sputtering [27][28]–[33].

2.4.4 Surface conductivity of adsorbed species

Zinc oxide has a strong sensitivity towards the exposure of its surface to various gases, which changes its conductivity. Zinc oxide based devices can be used as cheap smell sensors, to detect the freshness of the food and drinks based on the high sensitivity to trimethylamine present in the odor [34]. Based on the gas sensitive behavior of zinc oxide electrical conductivity (which increases in vacuum and decreases in normal atmosphere) it is possible to detect gases such as hydrogen, oxygen, and hydrocarbon. The sensitivity of this kind of sensors can be improved by doping [35]–[38]. The sensing capabilities are in strong correlation with the thickness of the material and the size of the structures (increasing when downscaling towards the nano-size[39], [40]. Table 3 presents zinc oxide based gas sensors, with and without dopants, and the gases detected.

Table 3. ZnO gas sensors

Sensor type	Dopant	Sensing Gas
single crystal	-	CO, CH_4
thick film	-	CO, H_2
thick film	undoped, Pd doped	NH_3
thick film	Sb	CH_4, H_2
pellet	Al	H_2
thin film (CVD)	-	H_2
thin film (sputter)	Al	NH_3
nanometer	Ag	CO, C_2H_2
nanometer	grain size	SF_6, C_2H_5OH

2.4.5 Optical properties

Comprehensive studies about the remarkable optical properties of zinc oxide show the possibility to use this material for a wide range of optoelectronics devices due to his direct wide bandgap at room temperature (E_g~3.3 eV), large exciton binding energy (60 meV) and efficient radiative recombination. The optical properties of semiconductor based materials lies in the presence of both intrinsic and extrinsic effects. The intrinsic optical transition takes place between the electrons in the conduction band and the holes in the valence band, including the excitonic effects caused by the Coulomb interactions. The extrinsic properties are related to dopants/impurities or point defects and complexes, which usually create electronic states in the bandgap and therefore influence both optical absorption and emission processes. The electronic states of the bound excitons (BEs) strongly depend on the semiconductor material, in particular the band structure. In theory, excitons could be bound to neutral or charged donors and acceptors. Other extrinsic transitions could be seen in optical spectra such as free-to-bound (electron-acceptor), bound-to-bound (donor-acceptor), and the so-called yellow/green luminescence (GL). The well-known green band in ZnO luminescence spectrum (manifesting itself as a broad peak around 500–530 nm) is observed nearly in all samples regardless of growth conditions and relates to singly ionized oxygen vacancies.

One of the unique properties of direct bandgap semiconductors is their ability to produce light emission in response to excitation mainly by means of electrical or optical injection of minority carriers. Light emission through any process other than blackbody radiation is called *luminescence* and requires external excitation, as it is a nonequilibrium process. When an external voltage is applied across a forward-biased p–n junction, as in the case of LEDs and lasers, electrons and holes injected into the medium from their respective ends recombine resulting in light emission called *electroluminescence* (EL). The emitted photon energy equals the difference of the energies of states occupied by electrons and holes prior to recombination. Another means of light emission, the *photoluminescence* (PL), is a result of incident-photon absorption that generates electron–hole pairs and produces emission of a photon of a different wavelength. The incident absorbed photons usually excite electrons from the valence band into the conduction band through momentum-conserving processes because the photon momentum is negligible. The electrons and holes thermalize to the lowest energy state of their respective bands via phonon emission before recombining across the fundamental bandgap or the defect levels within the bandgap and emitting photons of the corresponding energies. Photoluminescence (PL) measurements give important physical properties (defects of the lattice) for material characterization. Light emission can also be induced by raising the temperature of the semiconductor (thermoluminescence) and by subjecting the semiconductor to electron irradiation (cathodoluminescence) or other high-energy particle irradiation. Typical emission spectrum is rich with emission associated with intrinsic processes, meaning those not involving defects and impurities of any kind, and extrinsic processes meaning those involving impurities and defects in the form of either simple phases or complexes. Both intrinsic and extrinsic processes can be effectively studied by analyzing the steady-state PL, time-resolved PL (TRPL), and PL excitation (PLE) spectra.

For measuring the photoluminescence of zinc oxide typically a He-Cd laser is used. The schematic representation shown in Figure 3 depicts a typical PL measuring arrangement consisting from a light source (laser) for excitation, a dispersive element (grating monochromator), and a sensitive optical detector.

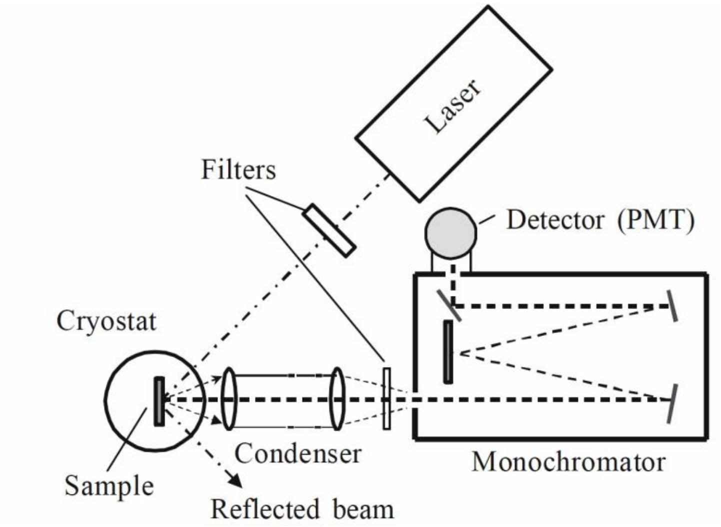


Figure 3. Schematic representation of a typical PL setup including a laser, a grating monochromator, detector (PMT), filters and collecting lenses.

Studies revealed that a high purity perfect zinc oxide crystal structure leads to a pure near band edge emission (NBGEE) at around 380nm [41], [42]. When the lattice of the zinc oxide is dominated by the presence of defects and impurities, a strong visible emission and a weak NBGEE can be observed. In Figure 4 a schematic diagram of the intrinsic defects in the lattice of zinc oxide materials is shown correlating the influence of oxygen vacancies (V_O), interstitial zinc (Zn_i), interstitial oxygen (O_i), zinc vacancies (V_{Zn}), substitution of Zn at O position (Zn_O) with the emission measured in the photoluminescence spectra [8]

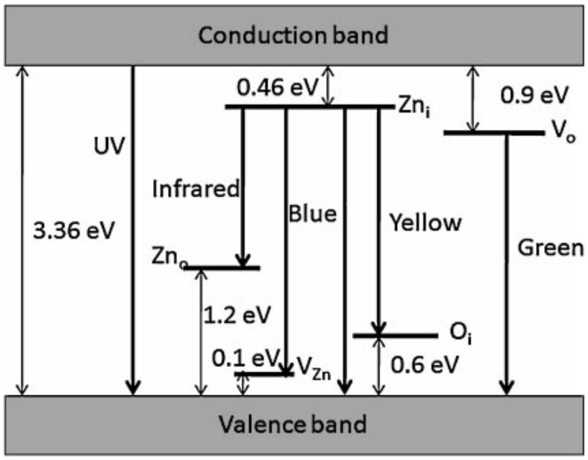


Figure 4. Schematic diagram of intrinsic point defect levels in ZnO [8].

Generally the UV emission at 380nm is attributed to the transition from the conduction band (CB) to the valence band (VB), blue emission isto the transition from the interstitial zinc Zn_i to the VB and the green emission is to the transition from the oxygen vacancies to VB [43], [44]. In Figure 5 typical PL emission spectra for different types of zinc oxide nanostructures such as tetrapods, needles, different kind of rods, shells and ribbons are shown.

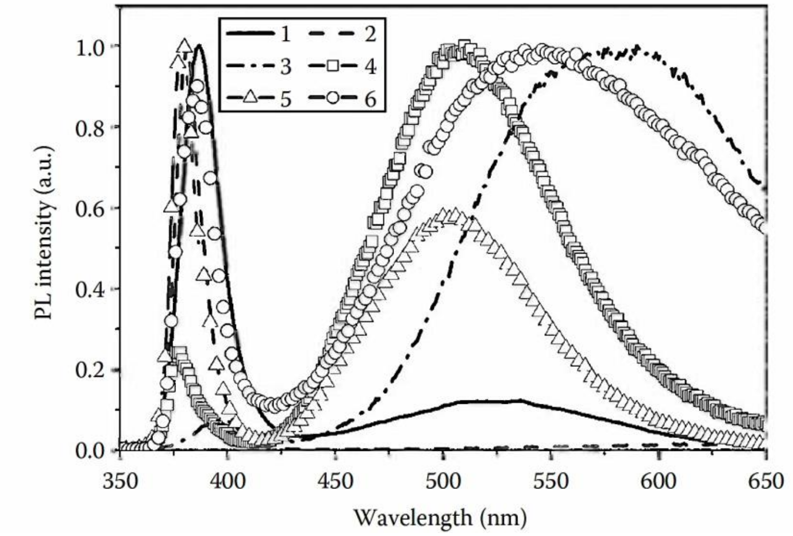


Figure 5. Example of room temperature PL spectra of various nanostructures in: (1) tetrapods, (2) needles, (3) nano-rods, (4) shells, (5) highly faceted rods, and (6) ribbons/combs [45].

2.4.6 Toxicity of zinc oxide

Zinc oxide has ceramic nature and thus can be used both as pigment and semiconductor. Zinc oxide exhibits antibacterial activity to a higher extent with Gram-positive B *subtilis* than towards the Gram-negative *E. coli* [46]. One of the most remarkable commercial applications of 20–100 nm ZnO nanoparticles is as sunscreens and cosmetics, due to their property of blocking broad UV-A and UV-B rays [47]. ZnO nanoparticles are believed to be non-toxic, bio-safe, and biocompatible nanomaterials [48], a very important aspect when developing bioapplications. Different studies performed on bacteria showed that low concentrations of zinc oxide nanoparticles did not induce any cellular damage [47][49] offering a promising base to develop new biocompatible applications

2.5 Zinc oxide nanostructures

Zinc oxide is a unique material that exhibits semiconducting, piezoelectric, and pyroelectricproperties. Using various synthesis methods and growth conditions zinc oxide can crystalize in a wide range of shapes (i.e. nanocombs, nanorings, nanohelixes, nanobelts, nanowires and so on). The nanostructures have and could have novel applications in optoelectronics, sensor industry, transducers, and biomedicine own to its biocompatibility.

Belt-like, quasi-one-dimensional nanostructures (called nanobelts) have been synthesized for semiconducting oxides of Zn, Sn, In, Cd, and Ga, by simply evaporating the desired commercial metal oxide powders at high temperatures. The as-synthesized oxide nanobelts were pure, structurally uniform, single-crystalline, and mostly free from dislocations; they have a rectangular-like cross-section with constant dimensions. The belt-like morphology appears to be a unique and common structural characteristic of this family of semiconducting oxides with cations of different valence states and materials of distinct crystallographic structures. Field-effect transistors [50], ultrasensitive nano-sized gas sensors [51], nanoresonators, and nanocantilevers [52] have been fabricated based on individual nanobelts.

Nananobelts, nanosprings, and nanorings [53], [54] with piezoelectric properties were synthesized for nanoscale transducers, actuators, and sensors.

Among the functional oxides with rutile, CaF_2, spinel, and wurtzite structures [55], zinc oxide is unique – it has semiconducting and piezoelectric properties in the same time. Zinc oxide is a material with diverse structures, and richer configurations than any known nanomaterial including carbon nanotubes. Controlling the growth kinetics, local growth temperature, and the chemical composition of the source materials, a wide range of nanostructures of zinc oxide have been synthesized as it can be seen in the examples in Figure 6.

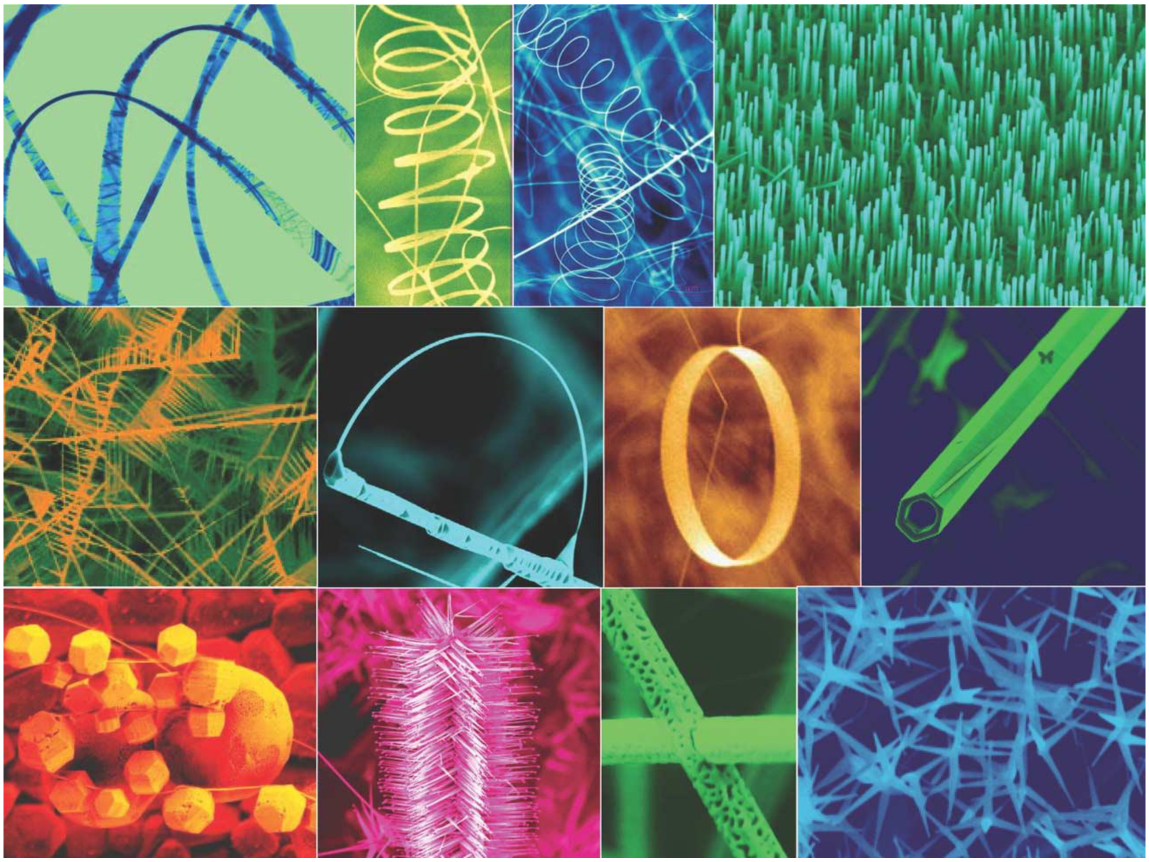


Figure 6. Zinc oxide nanostructures [56]**.**

2.6 Synthesis methods of zinc oxide

For most of its current applications ZnO is used in polycrystalline form, its crystalline quality or purity are not an issue. Advanced applications require single crystals in the form of bulk or thin films and a high degree of purity.

2.6.1 Bulk growth

Growth of bulk zinc oxide crystals is mainly carried out by three methods: hydrothermal [57]–[60], seeded vapor transport (sublimation) [61], [62], and melt growth [63], [64]. Because of its high vapor pressure, growth of ZnO from the melt is difficult, while vapor-phase deposition is difficult controled. A small amount of supersaturation of the solution during hydrothermal reaction fuels crystal growth. The hydrothermal method is very well known and established for the growth of quartz crystals thus suitable for the large-area ZnO single. (where anisotropic growth rate was reported [65])

2.6.2 Epitaxial growth methods

Device applications are interested in thin films and multilayers. The most common epitaxial deposition techniques used to synthetize semiconductor based materials are the radio frequency (RF) magnetron sputtering, molecular beam epitaxy (MBE), chemical vapor deposition (CVD), and pulsed laser ablation (PLA).

Pulsed lased deposition. The pulsed laser deposition (PLD) is a versatile technique to synthesize nanostructured materials. A high-power laser pulses are streamed on the surface of the bulk material and evaporates material from the target by ablation. PLD preserves the stoichiometry of the deposited material, an important advantage of this technique. Intensive studies were carried out over the years to understand how the ablation technique works [66]. The plasma plume formed during the ablation process expands away from the target with a strong forward-directed velocity distribution of different particles. The ablated species condense on the substrate placed opposite to the target. The main advantages of PLD are: it creates high-energy source particles, and it permits high-quality film growth at low substrate temperatures (typically ranging from 200 to 800℃). The simple experimental setup, and the possibility to operate in high ambient gas pressures (i.e. ranging between 10-5 –10-1 Torr) promote it as a cheap and suitable method for zinc oxide nanomaterials production. For the growth of zinc oxide by PLD technique, usually UV excimer lasers (KrF: $\lambda = 248$ nm, ArF $\lambda = 193$ nm) and Nd:YAG pulsed lasers ($\lambda = 355 \sim 1064$ nm) are used. Zinc oxide targets usually can consist of pressed powder of zinc or zinc oxide, or high purity targets are commercially available. The temperature of the substrate, the ambient oxygen pressure, and the laser intensity determine the properties of the obtained films. In Figure 7 a typical schematic of a pulsed laser deposition system is shown [67].

Thin zinc oxide films can been deposited on a wide variety of substrates (amorphous to single crystals), by PLD employing millisecond, nanosecond or femtosecond lasers. The pulse duration plays an important role in the crystalline state of the PLD-grown ZnO films.

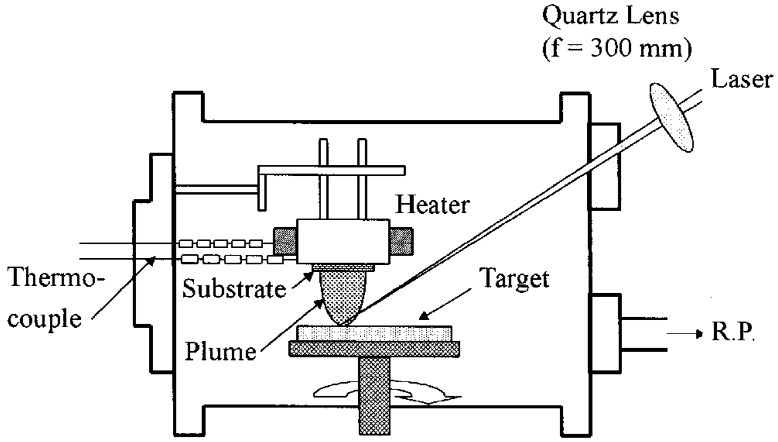
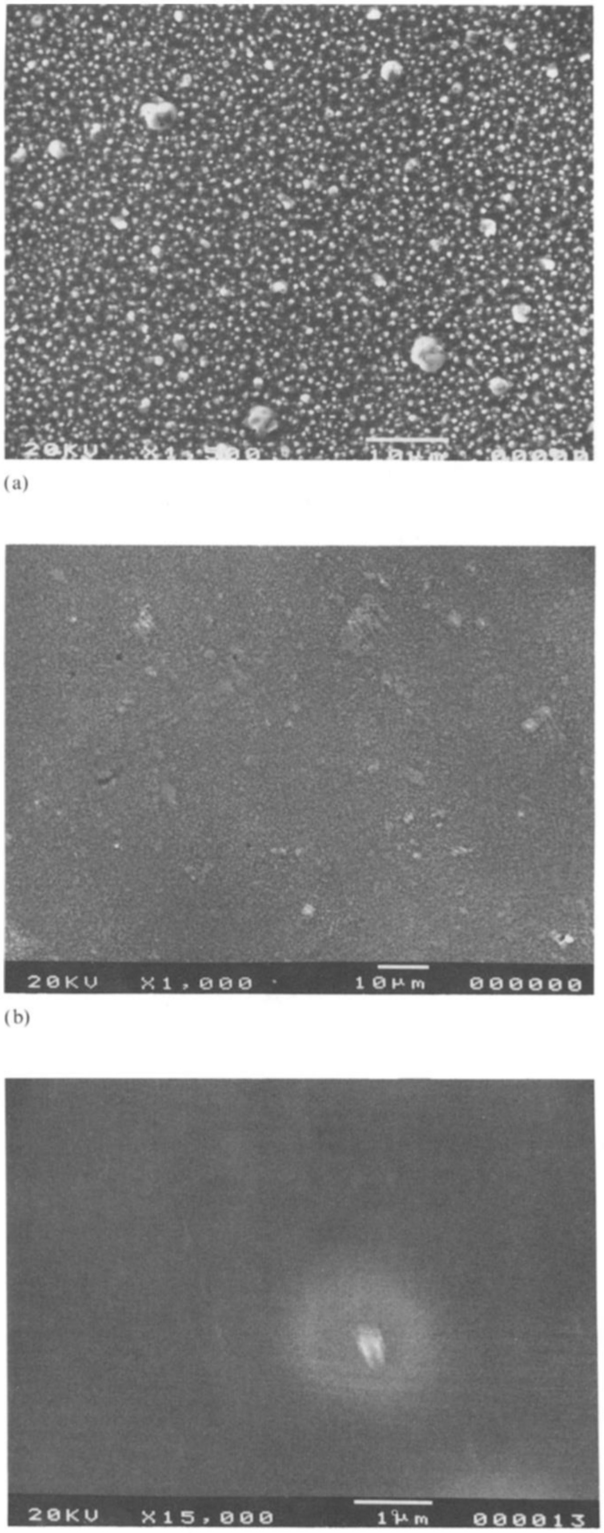


Figure 7. Schematic diagram of pulsed laser system [67].

Over the years many studies regarding the ablation of semiconductors were perform with different types of lasers. In the first report a tunable dye laser was used to investigate how the process works [68].

Zinc oxide deposited by 248 or 266 nm lasers exhibited a far superior morphology than films deposited with 532 or 1064 nm lasers, regardless of the experimental conditions as seen from the SEM images in Figure 8 where the structure of the thin film deposit using a 1064 nm laser consists from a high density of particles compared with 248 nm laser deposition which is almost featureless.



(a)

(b)

(c)

Figure 8. SEM images of zinc oxide films produced with lasers of wavelengths: (a) 1064nm, (b) 532nm, (c) 248nm [69]

Bionanotechnology and functionalization of the nanostructured materials

As a general definition, any structure with size of less than 100 nm is a true nanostructure, and unique phenomena are expected to take place at this scale. Nanotechnology is the concept that gives the possibility to manufacture and engineer at nanometer scale with atomic precision. Bionanotechnology is nothing else than a research branch of the nanotechnology where the inspiration for engineering at nanoscale is given by the biological processes that can be found everywhere around us.

When imaging biological processes, it is mandatory to make use of materials that firstly have the same size as the processes or structure to image, and secondly have some property that can be used for this purpose. Last but not least, they should be able to interact with the biological medium in such way as not to disturb the imaged process or structure. Therefore, special preparation of these nanomaterials needs to be done after their production.

The surface to volume ratio is much higher for nanomaterials than for microsize particles; the number of particles available per square unit of area in nano system is much higher than that for micron size system. This property makes nanoparticles suitable to act as catalysts or drug delivery systems. On the other hand, they are sensitive to the environmental conditions such as temperature, visible light, ultraviolet (UV), infrared (IR), pH, vibration forces, and so on. When dealing with nanoparticles, the atom-atom interaction determines the shape, size, geometry and orientation of the system, greatly contributing to the self-assembly property.

Gold, silver, zinc oxide, titania, and others have already been proven suitable for bioapplications. Even more, when these materials are functionalized with different structures, such as biomolecules or functional groups the nanomaterials can perform targeted delivery or can be used as biomarkers for detection of tumors (cancer therapy) [70], [71]. A schematic representation of a functionalized nanoparticle is presented in Figure 9 [72].

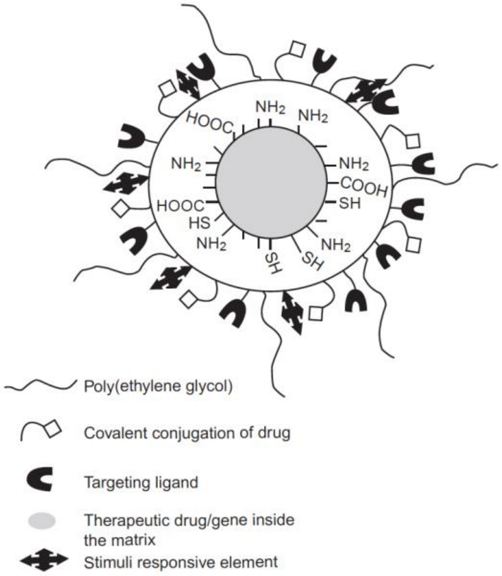


Figure 9. Schematic example of nanoparticles surface modification [72]**.**

3.1 Bioimaging

Biomolecular imaging (or *bioimaging*) is an important field for understanding the biological processes and for facilitating the interactions with biological targets. There are several noninvasive imaging techniques available for molecular imaging purposes, such as fluorescence imaging, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), ultrasonography [73]. All these techniques differ in terms of sensitivity, resolution, complexity, acquisition time, and operational cost. However, they are complementary as seen from Figure 10.

The imaging concept is used to define a variety of ways to define specific molecules present in living organism using externally activated agents or labeled particles that have the ability to sense specific molecular targets or cellular processes. Further studies are necessary to make a full understanding of the fundamental biological processes. New imaging methods could open the path for better differentiation in diagnosis and detection of diseases and help better monitor the therapies using bioimaging markers.

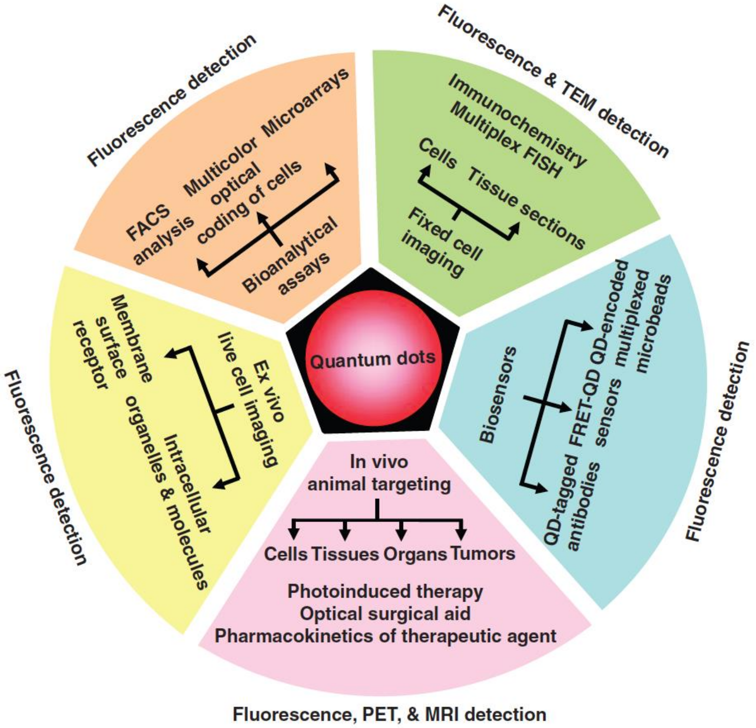


Figure 10. Applications of quantum dots as contrast agents in bioimaging [3]**.**

Recently the research has been focusing on the unique optical properties of quantum dots (QDs) in biological imaging. Optical bioimaging is based on traditional dyes, but there are several drawbacks associated with their use. It is well known that cell auto-fluorescence in the visible spectrum leads to the following effects:

- auto-fluorescence - can mask signals from labeled organic dye molecules,
- instability of organic dye under photo irradiation, which results in only short observation times,
- conventional dye molecules have a narrow excitation window, which makes difficult the simultaneous excitation of multiple dyes;
- dyes are sensitive to the environmental conditions, such as pH changes;
- most of the organic dyes have a broad emission spectrum.

Quantum Dots are of interest for several reasons:

✓ higher extinction coefficients,

- ✓ higher quantum yields,
- ✓ less photo-bleaching,
- ✓ absorbance and emissions can be tuned with size,
- ✓ generally broad excitation windows but narrow emission peaks,
- ✓ multiple quantum dots can be used in the same assay with minimal interference with each other,
- ✓ toxicity may be less than conventional organic dyes,
- ✓ quantum dots can be functionalized with different functional groups.

In semiconductors, the electronic ground level represented by the valence band is completely filled with electrons. The excited quantum states lie in the conduction band, which is empty and a minimum energy is necessary to excite an electron in a semiconductor; the energy released by de-excitation is equal to the band gap [74]. When a semiconductor absorbs a photon, an electron can be excited to a higher energy quantum state. If the excited electron returns (relaxes) to a lower energy quantum state by radiating a photon, the process is called *photoluminescence* (PL). Impurities and defects in the lattice act as activators and perturbe the band structure by creating local quantum states that might lie within the band gap. The predominant radiative mechanism in extrinsic luminescence is electron – hole recombination, which can occur via transitions between conduction band and acceptor state, donor state and valance band, or donor state and acceptor state.

Nanostructures possess optical properties that are highly dependent on the particle size or dimension. Such particles can be linked to biomolecules to form long-living sensitive probes for identification processes.

Figure 11 depicts CdS quantum dots of different sizes, the size correspondence to a different color, and also the emission spectra of CdS when excited by a UV source [75].

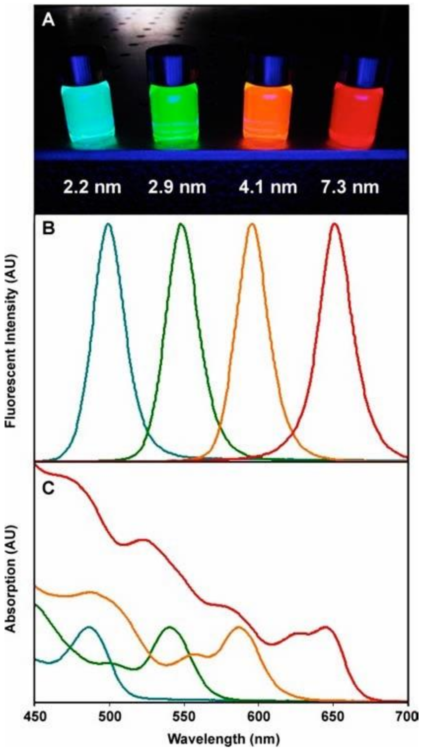


Figure 11. A) Fluorescent images of CdS QDs using a 365nm source. Sizes of QDs are 2.2nm (blue), 2.9nm (green), 4.1nm (orange) and 7.3nm (red). (B) Fluorescent spectra of the QDs exited with 400nm source. (C) Broad absorption spectra of the QDs. [75]

Quantum dots have already been used for live imaging of animal organs delivered through the intravenous route, demonstrating the ability of highly specific targeting. Different *in vivo* studies evaluate the efficiency of the quantum dots for targeted applications. Figure 12 shows green QDs conjugated to streptavidin, emitting green fluorescence. The image shows F-actins of fixed fibroblasts providing a high selectivity. The red fluorescent (CdSe)ZnS qunatum dots in Figure 13 were incubated with a monolayer of living human cancer cells.

Bioimaging with the use of nanostructures is an emerging technology in medical science having the great potential to revolutionize the way of diagnosis and treatment of diseases. By functionalizing different quantum dots with different functional groups and even biomolecules, new multicolor optical techniques can be developed. Current reports show the possibility to employ this kind of functionalized quantum dots for *in vivo* and *in vitro* imaging and diagnostic of live cell as it can be seen from Table 4.

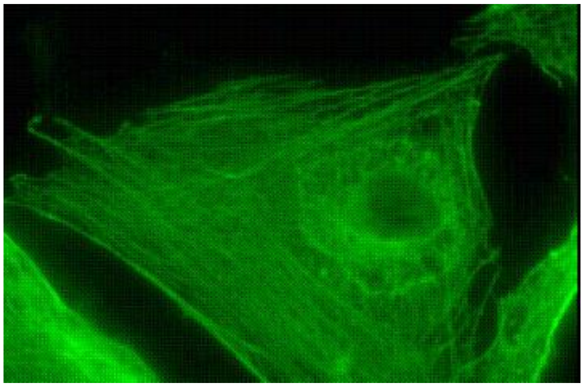


Figure 12. Immunocytochemical stain of F-actin in fixed fibroblast cells [76]

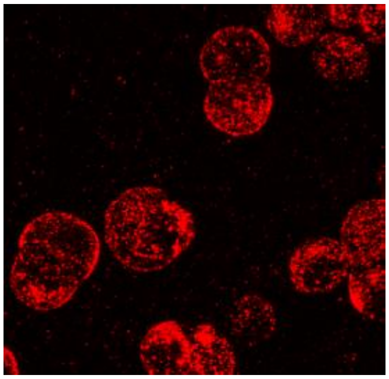


Figure 13 Human cancer cells incubated with (CdSe)ZnS QDs conjugated with TAT peptides [76]

Table 4. In vivo and In Vitro bioimaging studies using quantum dots

Quantum dots	Purpose	Imaging techniques	Reference
$CdSe/Cds/SiO_2$	Mouse fibroblast cell imaging	In vitro fluorescence	[77]
CdSe/ZnS	Biological detection	In vitro fluorescence	[78]
$CdSe/ZnS/SiO_2$	Phagokenetic track imaging	In vitro fluorescence	[76]
CdSe/ZnS	Tumor vasculature and lung endothelium imaging	In vitro and in vivo fluorescence	[76]
CdTe/CdSe	Cancer cell lymph nodes imaging	In vivo fluorescence	[79]
CdSe/ZnS	Maltose-binding protein	In vitro FRET	[80]

3.2 Functionalization of nanomaterials

Functionalization of surfaces through direct molecule attachment or by functional groups grafted on the surface is an important approach to tailoring the chemical, physical, and electronic properties of nanoparticle surfaces. By incorporating new functions on nanomaterials surface it's possible to develop devices and applications in different technological fields such as microelectronic computing, micro- and optoelectronic devices, micro-electromechanical machines, three-dimensional memory chips, silicon-based nano- or biological sensors, and nanopatterned organic and biomaterial surfaces. Dry reactions in vacuum and wet chemistry in solution are the two major strategies for functionalization.

A key motivation for the functionalization is achieving fine-tuning of the electronic properties. Chemical attachment of molecules to the semiconductor surface enables the necessary control over electron transfer through the semiconductor–organic interface providing a versatile and reproducible way to tailor the electronic properties of semiconductor surfaces in a controllable manner.

Functional groups are widely used for different types of materials starting from plastics to semiconductors. Functionalized materials exhibit unique chemical and physical properties and furthermore, if they are biocompatible, open new horizons for developing safe and accurate application for medical industry.

Functionalization of semiconductor surfaces is driven by significant technological requirements in areas including micro- and nanoscale electromechanical devices and development of new bioimaging techniques. In the last decades efforts in functionalization of semiconductor surfaces with organic materials and the subsequent immobilization of biospecies (such as DNA) at the surface were made leading to new developments for the biomedical industry. Figure 14 shows some of the recent progress of inorganic nanoparticles including their unique properties, surface functionalization strategies, and applications in biosensing and imaging therapeutics. Among them, reports describe the encapsulation of both magnetic nanoparticles and quantum dots within a silica shell to form a hybrid material denoted as SiO_2/MP-QD that has potential in bioimaging, drug targeting, biosensing, and biolabeling applications [81].

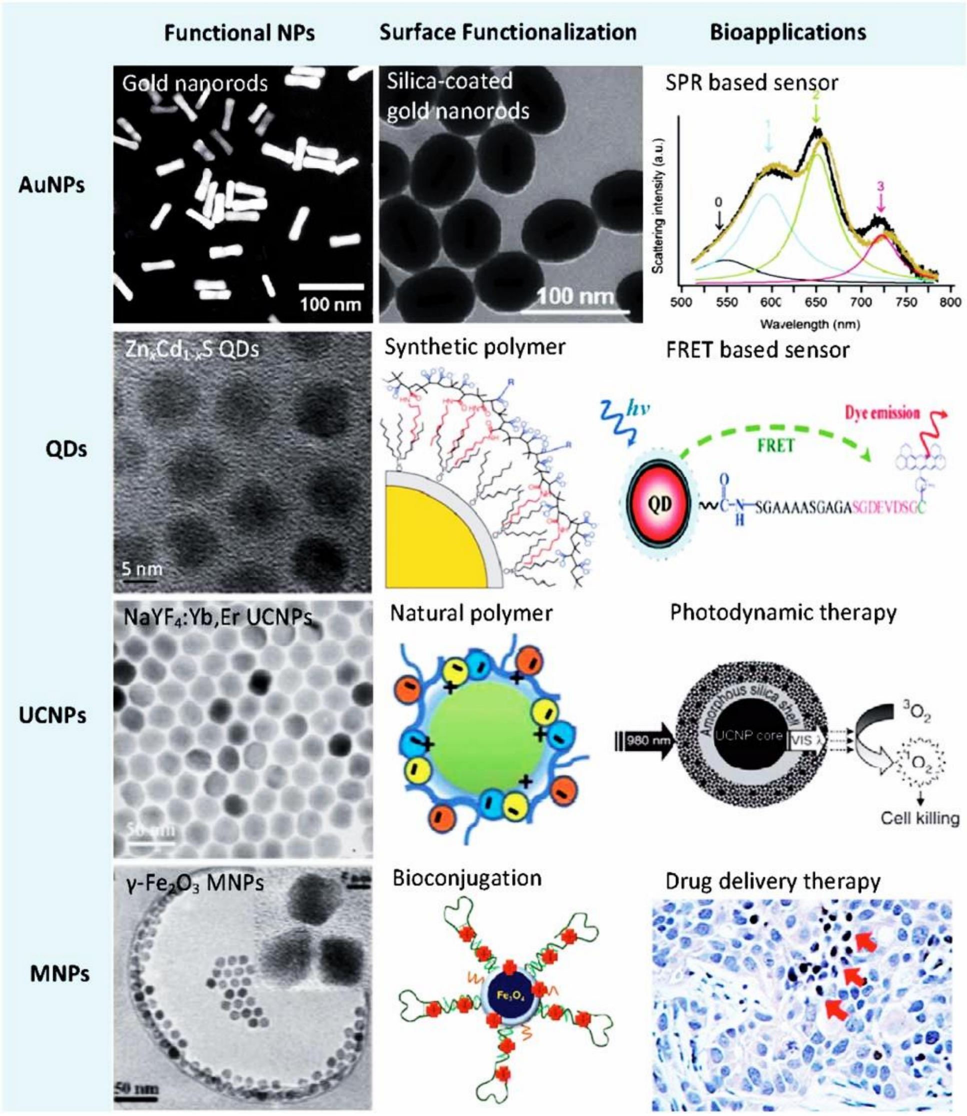


Figure 14. Surface functionalized nanoparticles: gold, quantum dots, upconversion nanoparticles and magnetic nanoparticles [81]–[83]**.**

To use the nanoparticles in bioapplications, their surface needs to be functionalized to provide stability, biocompatibility, and functionality. To appropriately tailor the surface of nanoparticles, chemists have designed different strategies to introduce the desired functionality on nanoparticles via surface encapsulation, in situ synthesis, or self-assembly. Currently, nanoparticles are widely functionalized with a variety of materials such as silica, graphene, synthetic polymers, biopolymers, dendrimers, and small molecules. In addition to tuning the surface properties, nanoparticles have been conjugated with biomolecules.

Surface engineering can tune the colloidal properties of nanoparticles to render them water-soluble and stable in physiological environments. In the next step, the surface-functionalized nanoparticles can be conjugated with biomolecules like peptides, proteins, or nucleic acids for developing interactions with living systems to participate in biological processes. The covalent binding of biomolecules with reactive primary amines, carboxylic acids and thiols on nanoparticles is a common bioconjugation method, which is suitable for various nanoparticles including gold, quantum dots, and magnetic nanoparticles [84], [85], [86].

3.3 Plasma processing of nanomaterials

Biocompatibility is a necessary property of materials during their interaction with biological systems; scientists and engineers who are dealing with medical and biological problems extensively investigate this aspect. It is a complex phenomenon and its mechanisms are not fully understood, mainly due to the lack of detailed knowledge of *in vivo* conditions. The degree of biocompatibility sets the limits to engineering solutions required for biomedical problems. Conventional wet-chemistry approaches employed for changing the physical–chemical characteristics of material surfaces often have application limitations owing to the complexity of the techniques, the requirement of environmentally non-friendly processes, and labor-intensive and expensive methodologies.

Non-equilibrium low and atmospheric pressure plasma techniques have been recognized for a long time as efficient surface modification techniques, and accordingly the plasma approach was also considered for the generation of biocompatible surfaces. Surface functionalization processes involving non-depositing plasma-gases, and plasma-enhanced deposition reactions have been investigated for the modification of biomaterial surfaces.

Plasma-surface modification is an effective and economical surface treatment technique for many materials and of growing interests in biomedical engineering. The unique advantage of plasma processing is that the surface properties and biocompatibility can be selectively enhanced, thus enhancing the performance of the nanomaterials. By altering the surface functionality using plasma processing the optimal surface, chemical, and physical properties can be attained. Surface modification by plasma, especially those biocompatible with the living tissuea, is becoming an increasingly popular method to improve device multifunctionality, tribological, and mechanical properties, as well as biocompatibility of artificial devices. By plasma processing, the nanomaterials can be cleaned, etched and/or activated.

Plasma is regarded as the fourth state of matter and is composed of highly excited atomic, molecular, ionic, and radical species. It is typically obtained when gases are excited into energetic states by radio frequency (RF), microwave, or electrons from a hot filament discharge. Plasma is a highly reactive chemical environment; any surface introduces inside plasma will be subjected to many plasma-surface reactions. The high-density of ionized and excited species in the plasma can change the surface properties of normally inert materials. Plasma-based techniques combining the advantages of conventional plasma and ion beam technologies are effective methods for medical technologies and by changing the surface energetics of the nanomaterials, improvements of the adhesion strength, surface and coating properties and biocompatibility can be achieved.

The key points that promote plasma technology as suitable processing methods for nanomaterials are as follow:

- ✓ **Since the discovery of plasma many studies were conducted for understanding the physics and chemistry of the process.**
- ✓ **Plasma engineering already proved to be a reliable, reproducible, relatively inexpensive and applicable to different sample geometries as well different materials such as metals, semiconductors, polymers, ceramics and composite materials.**
- ✓ **Plasma processes can be accurately and in real time monitored using commercially available in situ plasma diagnostic devices.**
- ✓ **Plasma treatment can result in a variety of changes of the surface characteristics of nanomaterials: chemical, tribological, electrical, optical, mechanical and biological.**

- ✓ **Plasma processing is a technique that can be easily implemented in industrial applications.**

Plasma cleaning property already promoted this technique as an effective, economical, and environmentally safe method for critical surface preparation. It was demonstrated that plasma cleaning eliminates natural and technical oils and grease at the nano-scale and reduces contamination up to six fold when compared with traditional wet cleaning methods, including solvent cleaning residues themselves. Plasma cleaning produces a pristine surface, ready for bonding or further processing, without any harmful waste material. In this case the cleaning effect is due not only to the ultra-violet light generated in plasma but also to the reactive species able to react with the organic contaminants to form mainly water and carbon dioxide which are continuously removed (pumped away) from the chamber during processing. With plasma activation this effect is increased because reactive species from the plasma bind to active surface sites all over the material, creating a surface that is highly 'active' to bonding agents.

Plasma polymerization is another way to modify material surfaces. In plasma polymerization, the transformation of low-molecular-weight molecules (monomers) into high-molecular-weight molecules (polymers) occurs with the assistance of energetic plasma species such as electrons, ions, and radicals.

Plasma contains activated species able to initiate chemical and physical reactions at the solid surface of the nanomaterials and as a result changes of the surface properties and surface morphology occur. The species in plasma that lead to the process of plasma treatment are electrons, ions, and radicals and they are generated by the collision between electrons and the existing gas molecules

The most important process for surface modification of the nanomaterials is represented by the implantation reactions. Gas molecules are activated by plasma and created species inside plasma interact with the surface of the materials and special functions such as hydroxyl, carboxyl, carbonyl and amino groups are bonded at the surface of the nanoparticles. The creation of these functional groups is in strong correlation with the mixture of gases used to ignite the plasma.

For biomedical and sensing fields is of great interest the development of nanoparticles with functional groups such as carboxyl (-COOH), amines (-NH_2), hydroxyls (-OH), and aldehydes (-CHO).

Aminated surfaces have been fabricated using various plasma vapors or mixtures (allylamines, ethylenediamine, diaminopropane, ethylamine [87]) and have found wide use for biointerface applications. Plasma-fabricated surfaces that contain carboxyl groups have also been well documented by employing acrylic acid [88] and vinyl acetic acid or applying CO_2 plasma.

Figure 15 depicts the process of surface functionalization of the graphite-encapsulated iron nanoparticles using plasma treatment as an important step toward the immobilization of biomolecules. Plasma processing of nanomaterials gained a lot of attention in the last decades due to the versatility of the method offering a viable alternative to the wet chemical methods widely employed.

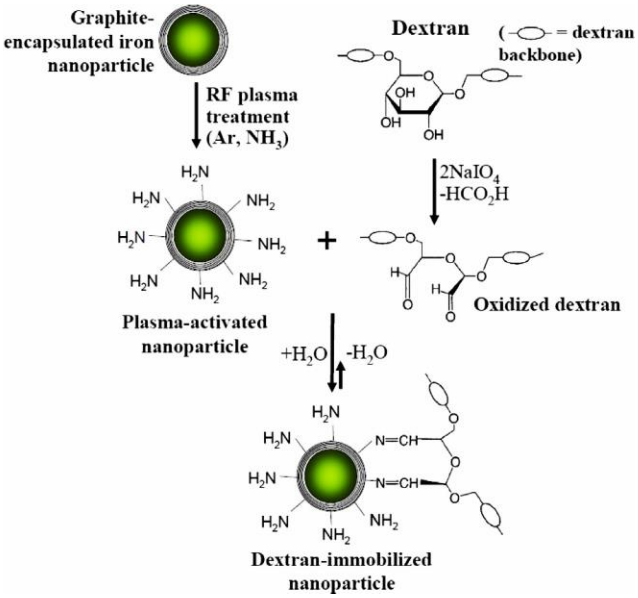


Figure 15. Plasma surface modification of graphite-encapsulated iron naoparticles followed by covalent immobilization of dextran [84]**.**

3.4 Functionalization of zinc oxide nanomaterials

As a nanomaterial, zinc oxide has attracted the interest over the past several years and has become one of the most popular semiconductor based material. Moreover, due to its unique optical properties combined with antibacterial and low toxicity many reports promotes it as an alternative to other materials long used in biomedicine field and which are toxic for the living organisms.

Multifunctional zinc oxide and its nanostructures possess unique properties that are suitable for biosensor technology. Zinc oxide based sensors have demonstrated high sensitivity to various gases and liquids such as CH_4, CO, H_2O, H_2, NH_3, trimethylamine, ethanol, and NO_2 [89]. Zinc oxide and its ternary alloy, $Mg_xZn_{1-x}O$, are known to be the biocompatible oxides, in which Zn and Mg are important elements for neurotransmitter production and enzyme function [90]. Zinc oxide nanostructures are also employed as a coating materials for medical implants [91]. ZnO nanostructures are compatible with intracellular media and are highly sensitive to pH changes inside cellular environments [92] with $Mg_xZn_{1-x}O$ having a large range of pH compatibility. Zinc oxide nanorods are also used for detection of enzymatic reactions [93]. The biocompatibility of zinc oxide and its feasibility for biosensing applications were demonstrated in detections of proteins, antibodies, and DNA through the proper surface functionalizations [94]. The control of the surface wettability of zinc oxide nanotips between the superhydrophilic and superhydrophobic states is used to dramatically enhance the sensitivity of the biosensors [95].

Intracellular pH sensing was reported by employing zinc oxide nanorods coated on silver coated capillary glass and Ag/AgCl microelectrodes. The Ag/AgCl electrode was used as reference and by measuring the electrochemical potential difference between the two electrodes pH was determined. The sensor is used to measure intracellular *p*H in a single human fat cell and the results demonstrated the capability of performing biologically relevant measurements inside living cells [96].

Zinc oxide can also serve as the basic sensing platform for detection of biophysical activity of larger biospecies such as cells, bacteria, and viruses. The variation in morphology of the zinc oxide nanostructured surfaces impacts the attachment of certain biological cell lines (for example fibroblasts, umbilical vein endothelial cells, and capillary endothelial cells) and control the extent of cellular adhesion [91]. Zinc oxide nanostructures were also used to bind with bacterial and viral cultures for reaction with enzymes and antibodies for applications in immunosensing [97] and have been employed in intracellular measurements where zinc oxide nanotips were used as coatings for microprobes or served as the probes themselves. These intracellular probes can sense conductivity changes due to the zinc oxide coating reaction to various target biochemicals within the cellular environment. Microtubes coated with ionophore-functionalized zinc oxide nanorods were used as an intracellular probe to selectively sense the Ca^{2+} ions within the cell membrane [98]. Different methods were reported for determining the membrane potential across a human fat cell [99]

Reports about zinc oxide nanorods being employed as fluorescence enhancers proved the possibility to achieve high detection sensitivity of sub-picomolar and attomolar levels using a conventional fluorescence microscope [100]. Zinc oxide has also been reported to increase the detection of optical signal in Raman spectroscopy in the Escherichia coli when the nanoparticles were introduced into the cells [101].

The design of a smart multifunctional ZnO-based biosensing platform is due to three important components of ZnO's properties:

① **reversible wettability control,**

② **bi-functional biochemical binding (serves as both active biological attachment and optical platform, i.e., fluorescence emission),**

③ **nanostructure morphology control.**

Biofunctionalization is a critical issue for ZnO nanostructured surface to obtain the high sensitivity and selectivity to various types of biospecies. The binding properties of a number of small molecules with functional groups are reported to form covalent bonds with metal oxides nanoparticles, including ZnO [102], COOH, SH, $SiOMe_3$, SiCl3, and $PO(OH)_2$ as depicted in Figure 16.

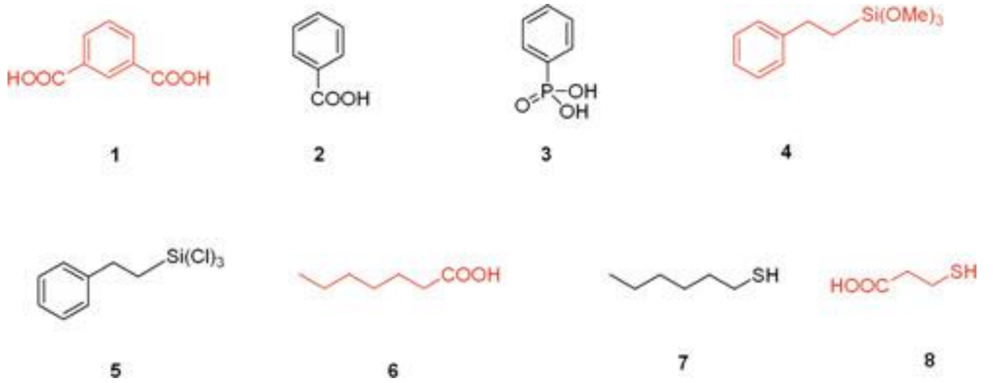


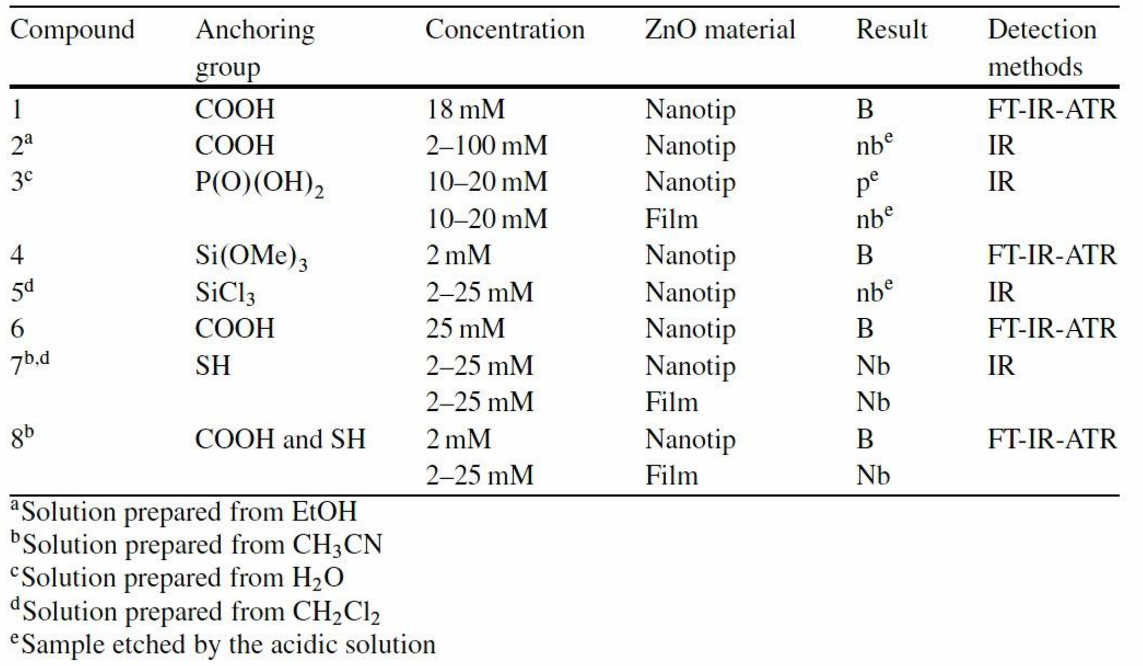
Figure 16. Different compound used for biofunctionalization of zinc oxide.

To achieve the selective biochemical attachment to the zinc oxide nanostructures bifunctional linkers are employed, having one functional group that attaches to the zinc oxide surface while leaving other end available for further biological functionalization as it can be seen from Table 5 [103]. This table offers detailed information on how the functional group is attached on the surface of the zinc oxide nanostructure, how it can be detected, and also the preparation methods for the functionalized zinc oxide. The ability to functionalize the zinc oxide nanostructures would lead to numerous applications, such as ZnO-based integrated, multimode, and multifunctional sensor technology to achieve higher accuracy and selectivity than existing sensors.

Surface functionalization of zinc oxide for development of biosensors that can be integrated with microelectronics was reported by employing long chain carboxylic acids linkers to connect single-stranded DNA with the zinc oxide [94]. The methods describe a stepwise functionalization of zinc oxide that allows sequential reactions on the surface of the nanostructures. By employing long chain bifunctional linkers that bind to zinc oxide, the terminal carboxylic group represents a reactive site that can form covalent bonds with other biomolecules.

The process of functionalization of zinc oxide based nanostructures was described in many reports over the years and important breakthroughs towards the development of new techniques, which can deal with biological processes, can be nowadays achieved.

Table 5. Compounds bounds [b], physisorbed [p] or nonbound [nb] to zinc oxide nanostructures [103]**.**



Compound	Anchoring group	Concentration	ZnO material	Result	Detection methods
1	COOH	18 mM	Nanotip	B	FT-IR-ATR
2[a]	COOH	2–100 mM	Nanotip	nb[e]	IR
3[c]	$P(O)(OH)_2$	10–20 mM	Nanotip	p[e]	IR
		10–20 mM	Film	nb[e]	
4	$Si(OMe)_3$	2 mM	Nanotip	B	FT-IR-ATR
5[d]	$SiCl_3$	2–25 mM	Nanotip	nb[e]	IR
6	COOH	25 mM	Nanotip	B	FT-IR-ATR
7[b,d]	SH	2–25 mM	Nanotip	Nb	IR
		2–25 mM	Film	Nb	
8[b]	COOH and SH	2 mM	Nanotip	B	FT-IR-ATR
		2–25 mM	Film	Nb	

[a] Solution prepared from EtOH
[b] Solution prepared from CH_3CN
[c] Solution prepared from H_2O
[d] Solution prepared from CH_2Cl_2
[e] Sample etched by the acidic solution

Even so there are still many challenges regarding the use of this kind of nanoparticles as feasible commercial applications. As shown in this chapter the functionalization of the materials is widely achieved by employing wet chemical techniques that still have huge issues such as particle agglomeration, low stability of the processed nanomaterials, and not to forget the toxicity that can arise from the use of dangerous and complex chemicals.

3.5 Plasma functionalization of the surface of zinc oxide

Until now, as previously stated, the functionalization of zinc oxide based materials in different shapes and sizes has been exclusively achieved by chemical methods. There is a lack of reports regarding surface preparation of zinc oxide nanomaterials for bioapplications. In this study, we propose for the first time a novel approach to functionalize the zinc oxide nanostructure surface employing plasma technologies that have been proving their benefits in the materials processing industry for a wide range of nanomaterials.

Experimental methods

4.1 Methods of investigation and preparation of nanostructured materials

4.1.1 X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS), also known as Electron Spectroscopy for Chemical Analysis (ESCA), is a widely used analytical technique for investigating the chemical composition of solid surfaces. The quantification by X-ray photoelectron spectroscopy (XPS or ESCA) is based on the Einstein's explanation of the photoelectric effect [104].

Surface analysis by XPS involves the irradiation of the solid with monoenergetic soft X-rays and sorting the emitted electrons by energy. By plotting the results, the obtained spectrum depicts the number of emitting electrons per energy interval versus their kinetic energy (or binding energy). Most common X-ray sources are represented by MgK_α X-rays (1253.6 eV) and AlK_α (1486.6 eV). Each element has an unique elemental spectrum, and the spectral peaks from a mixture are approximately the sum of the elemental peaks from the individual constituents. The limitation of the technique appears only from the very small mean free path of the electrons and thus the detected species originate only from the top atomic layers, more precisely from maximum 10 nanometers of the surface. Quantification of the data is possible through the peak heights or areas and identification of chemical states can often be made from the exact position and separation of the peaks.

The kinetic energies of the emitted electrons due to the photoelectric effect is given by the equation:

$$KE = h\vartheta - BE - \phi_s$$

where $h\vartheta$ is the energy of the photon, BE is the binding energy of the atomic orbital from which the electron originates, and $\phi_s$is the spectrometer work function.

To analyze our samples, we used Shimadzu ESCA-3400 with an Mg anode, system presented in Figure 17. Survey spectra along with high-resolution spectra for the elements of interest (i.e. Zn 2s, Zn 2p, O 1s, N 1s) were recorded and subsequently interpreted with OriginLab Analysis suite. All the measurements were performed in ultra high vacuum ($<5\times10^{-6}$ Pa) to remove contamination from the ambient atmosphere.

Figure 17. ESCA-3400 device image used for XPS measurements.

4.1.2 Transmission Electron Microscopy (TEM)

The fine structure of the materials was analyzed with transmission electron microscope (TEM), which provides information not only on the shape and size of the structures but also on their crystallinity.

In the current study High Resolution Transmission Electron Microscopes (HRTEM) (JEOL 2100) is employed for the characterization of zinc oxide nanoparticles. TEM analysis provides the information necessary to optimize the growing mechanism in the case of our lab-made particles, and gives info about the other nanomaterials used in the study.

TEM device consists of an electron gun, which produces an electron beam confined by condenser lens. After passing through the sample (as seen from Figure 18) the objective lenses project the image onto the phosphorescent screen or CCD camera. Several apertures are located between the sample and the screen to select the elastically scattered electrons that form the image.

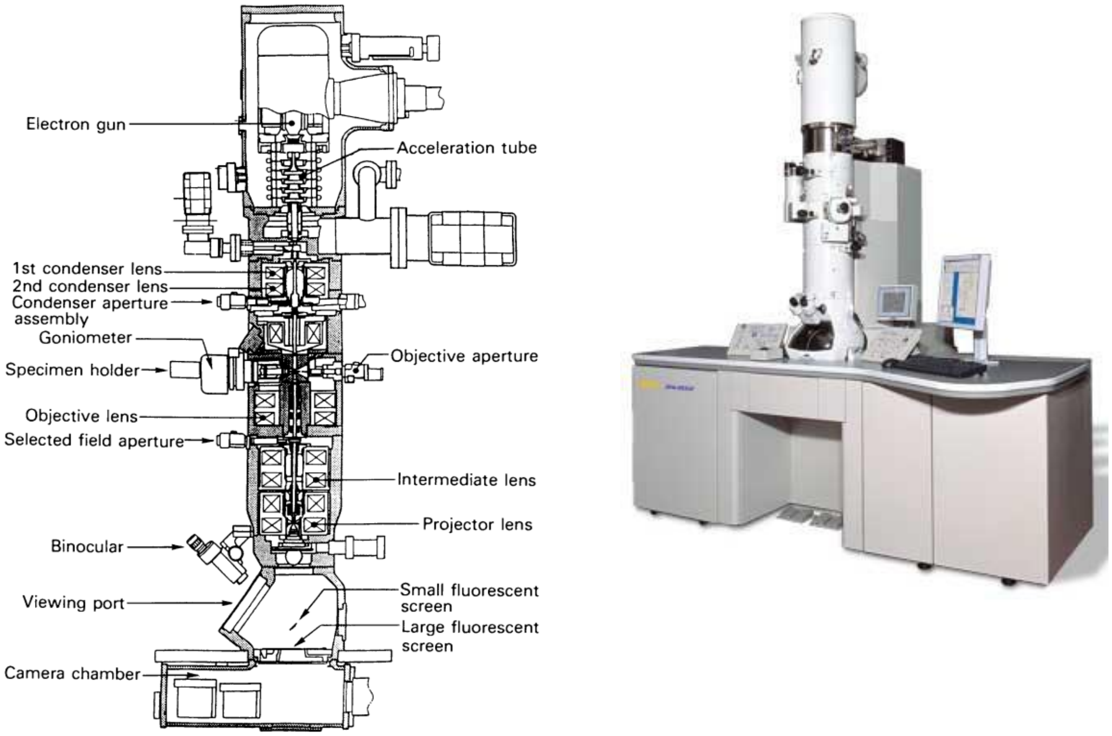


Figure 18. (left)Schematic drawing of a TEM device and (right) JEOL2100F instrument.

4.1.3 Chemical Derivatization – Nynhidrin Assay

Nynhidrin reactions are widely used to analyze and characterize amino acids, peptides, and proteins as well as numerous other ninhydrin-positive compounds in biomedical, clinical, food, forensic, histochemical, microbiological, nutritional, and plant studies.

To confirm the fact that by plasma processing we introduce amine groups on the surface of our zinc oxide nanostructures, the nynhidrin assay is employed. Ninhydrin connects amine and amide groups; due to this reaction a purple compound (in the case of amines reaction) or a yellow compound (in the case of amine reaction) is released. Furthermore, quantification of the functional groups can be achieved by measuring with a spectrophotometer absorbance at 570nm, and 440nm, respectively. The absorbance measured at 570 nm is proportional with the number of amine functionalities, while the absorbance at 440 nm indicates the number of amide functionalities.

The assay implies the preparation of a citrate buffer with a molar concentration of 0.1 M and a pH of 5. Citrate buffer, zinc oxide sample, and the derivatization reagent represented by ninhydrin are mixed in a solution and then incubated at 100℃ for 15 minutes as schematically presented in Figure 19. Afterwards, the absorbance for the two abovementioned wavelengths are determined.

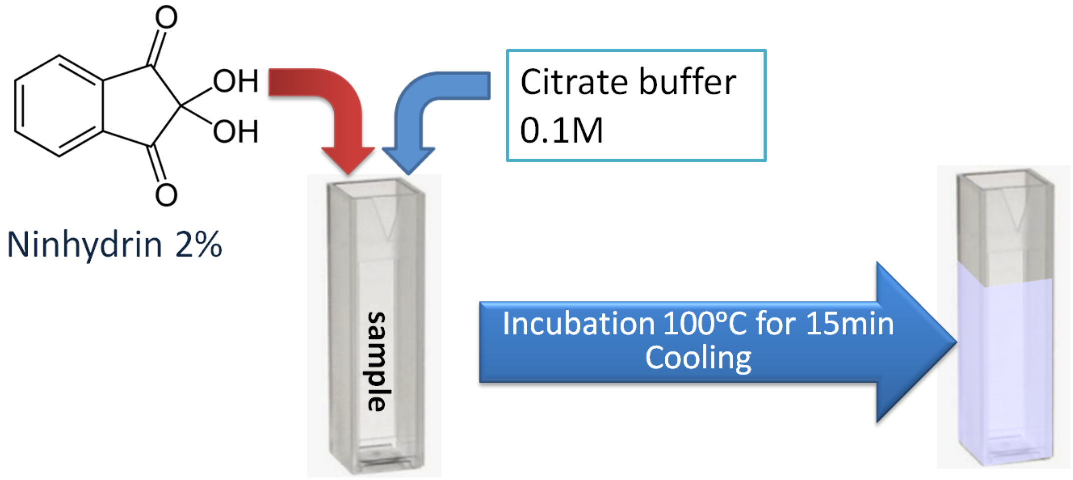


Figure 19. Ninhydrin derivatization procedure.

4.1.4 Fluorescence microscopy

A fixed and largely featureless preparation of nanomaterial may reveal a lot of its structure if stained with a proper dye – a substance that recognizes specifically structure and absorbs light of a selected wavelength. Fluorescent materials absorb light in a specific wavelength range, and re-emit it with lower energy, that is, shifted to a longer wavelength. Today, a very large number of different dyes with absorption from the UV to the near-infrared region are available and the principal advantages of this approach are a very high contrast, sensitivity, specificity, and selectivity.

The most attractive feature of fluorescence microscopy is the ability to image and study quantitatively not only the structure but also the function, that is, physiology of intact cells in vitro and in situ.

In Figure 20 a schematic diagram of a fluorescence microscope is depicted with the constituent parts: halogen lamp (HL), objective lens (OB), exciting light source (EXL), collector lens (CL), dichroic mirror (DM), excitation filter (EXF), emission filter (EM).

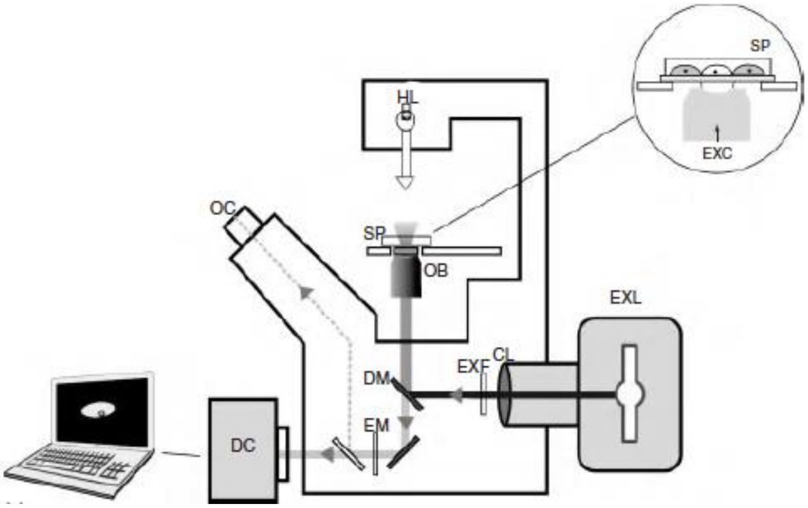


Figure 20. Schematic diagram of a fluorescent microscope.

Fluorescence is excited by light emitted by a mercury lamp. The exciting light is reflected toward the sample by a dichroic mirror that reflects the chosen exciting wavelength and allows the passage of the expected fluorescence as it can be seen from Figure 21.

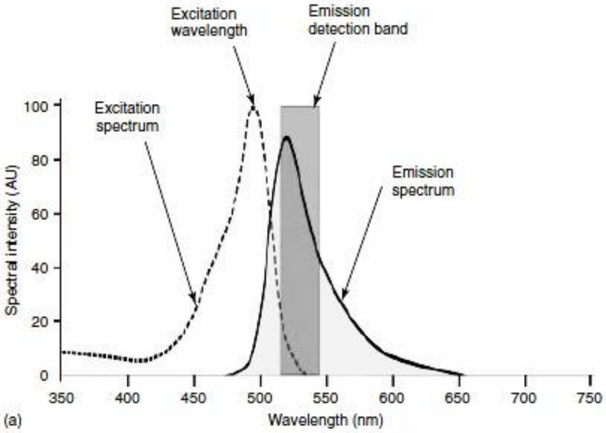
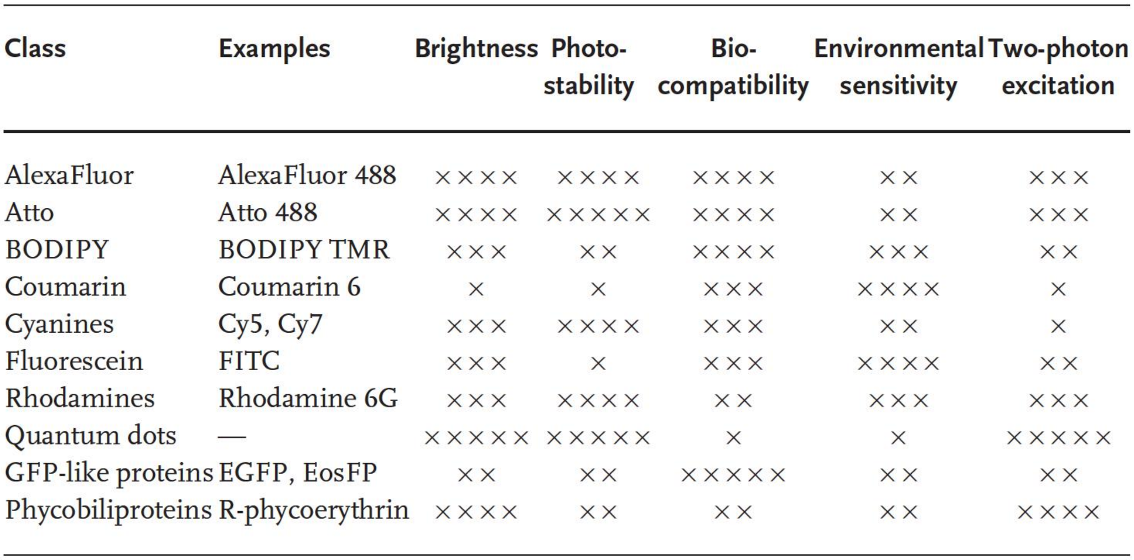


Figure 21. Spectral properties of a typical fluorescent label.

The potential of fluorescence imaging is to a great extent determined by the physicochemical properties of the fluorophores employed, including chemical nature and size, optical properties, biocompatibility, and interplay between dye and biological unit. In general, there is a large variety of fluorophores to choose from. In Table 6 a comparison of some fluorescent markers with their key properties is presented.

Table 6. Fluorescent markers.



Class	Examples	Brightness	Photo-stability	Bio-compatibility	Environmental sensitivity	Two-photon excitation
AlexaFluor	AlexaFluor 488	××××	××××	××××	××	×××
Atto	Atto 488	××××	×××××	××××	××	×××
BODIPY	BODIPY TMR	×××	××	××××	×××	××
Coumarin	Coumarin 6	×	×	×××	××××	×
Cyanines	Cy5, Cy7	×××	××××	×××	××	×
Fluorescein	FITC	×××	×	×××	××××	××
Rhodamines	Rhodamine 6G	×××	××××	××	×××	×××
Quantum dots	—	×××××	×××××	×	×	×××××
GFP-like proteins	EGFP, EosFP	××	××	×××××	××	××
Phycobiliproteins	R-phycoerythrin	××××	××	××	××	××××

In our study we used a fluorescent dye (Alexa Fluor 488) that can specifically connect to the ammine groups and can be visualized with the fluorescence microscope. Same method was involved when detecting whether labeled sugar chains connected the amine functionalities on the surface of the functionalized zinc oxide nanomaterials.

4.1.5 Chemical derivatization for quantification of amine groups number grafted on the surface of zinc oxide by plasma processing – SPDP protocol

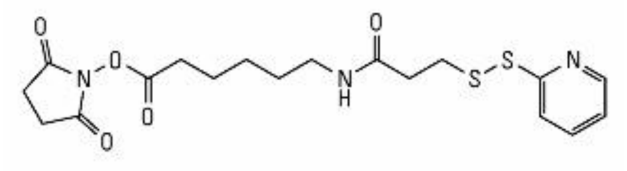
Crosslinking is the process of chemically joining two or more molecules by a covalent bond. The technique, often called bioconjugation when referring to its use with proteins and other biomolecules, is an essential component of many quantification methods and strategies for investigating functional materials structure and interactions. Crosslinking reagents (or crosslinkers) are molecules that contain two or more reactive ends capable or chemically attaching to specific functional groups (primary amines, sulfhydryls, etc.) on nanostrucrures or other molecules.

A number of chemical reactive groups have been characterized and used to target the main functional groups as seen from Table 7. Many different crosslinking reagents can be synthesized when different combinations of two or more of these reactive groups are incorporated into one molecule.

Table 7. Popular crosslinker ractive groups [105]**.**

Reactivity Class	Chemical Group
Carboxyl-to-amine reactive groups	Carbodiimide (e.g., EDC)
Amine-reactive groups	NHS ester Imidoester Pentafluorophenyl ester Hydroxymethyl phosphine
Sulfhydryl-reactive groups	Maleimide Haloacetyl (Bromo- or Iodo-) Pyridyldisulfide Thiosulfonate Vinylsulfone
Aldehyde-reactive groups	Hydrazide Alkoxyamine
Photoreactive groups	Diazirine Aryl Azide
Hydroxyl (nonaqueous)-reactive groups	Isocyanate

For the quantification of the amino groups bonded on the surface of our zinc oxide nanoparticles we used Sulfo-LC-SPDP (sulfosuccinimidyl 6-[3(2-pyridyldithio)propionamido]hexanoate) from Thermo Scientific (Figure 22).



LC-SPDP

Succinimidyl 6-[3(2-pyridyldithio)propionamido]hexanoate

MW 425.52

Spacer Arm 15.7 Å

Figure 22. Chemical Sructure of Sulfo-LC-SPDP crosslinking reagent.

The quantification of amine groups on the surface of the zinc oxide nanoparticles was performed as described in Figure 23. The SPDP crosslinkers connect with the amine groups through a reaction performed under dark conditions and ultrasonication for 30 min. The new created bonds are the cleaved with the help of a reducing agent, in this case DTT (Dithiothreitol). In this way, one Pyridine 2-thione molecule is released from each SPDP molecule connected to one amine group in the sample. Estimating the number of pyridine molecules leads to the quantification of amine functionalities on the surface of our samples.

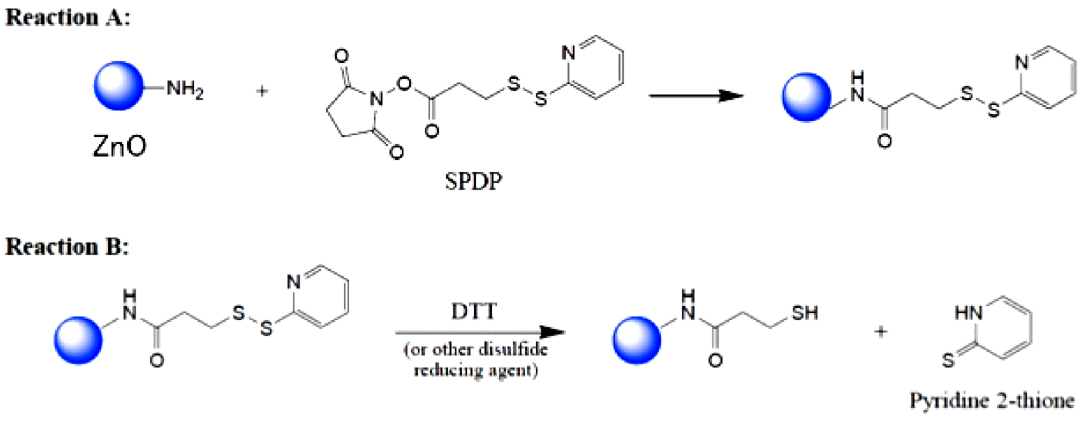


Figure 23. Chemical derivatization reactions involved in the procedure of amine group quantification for aminated zinc oxide nanoparticles.

Pyridine is a chromofore and thus, its absorbance at 343nm can be used to calculate the number of amine groups in the samples. Relating with the Beer-Lambert law the the molar concentration of the absorbing species can be calculated through the formula:

$$\Delta A = \varepsilon l c$$

where ε is the molar absorptivity of the absorber, l the distance the light travels through the solution and c the molar concentration of absorbing species.

4.1.6 Photoluminescence properties

As previously described, the photoluminescence of the zinc oxide is of great interest and we aim to take it into consideration for the development of new imaging techniques.

For the current studied, we used an experimental device schematically shown in Figure 3 zinc oxide is excited with a He-Cd laser having a wavelength of

325 nm and the photoluminescence spectrum is recorded with a photomultiplier (Hamamatsu Photonics).

More details about photoluminescence measurements will be discussed in the results section.

4.2 Zinc oxide nanoparticle production and processing

4.2.1 Pulsed laser deposition

As described in Chapter 1, pulsed laser deposition is a widely used technique for the production of nanostructured materials. In this study we want to make use of our previous experience regarding the zinc oxide fabrication and prepare nanostructures suitable for bioapplications using pulsed laser ablation technique.

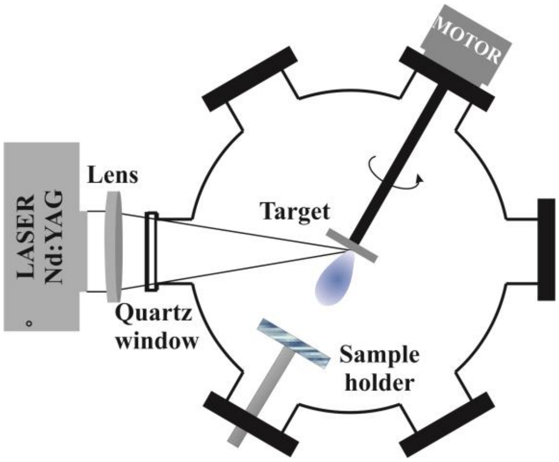


Figure 24. Schematic drawing of the vacuum chamber used in the laser ablation technique[106]**.**

In the experiments presented here we used Nd:YAG laser with a wavelength of 1064 nm to ablate commercially available pure zinc oxide targets (99.999% purity) with a diameter of 3 centimeters (see Figure 24). The ablation process takes place in a pure reactive oxygen atmosphere and the energy of the laser unit is kept constant at around 7 J/pulse. Surface wave excited plasma processing

4.2.2 Surface wave excited plasma processing

Gaseous discharges have been known as sources of electrons and other active species such as neutral, ionized and excited atoms and molecules as well as radicals and they have been used diverse chemical processing, as ion sources and so on. The fastest expanding area of their application seems to be the treatment of the surfaces: processing of semiconductors (deposition, oxidation, etching, and encapsulation), nitridation of the metal surfaces and dielectric coating.

The concept of surface waves dates since 1960s [107] but their application for the plasma excitation appeared several years later in 1970s when the first surfactron structure plasma source was designed. Over the years improvements of the early design were made to be able to enlarge the discharge area. This was possible mainly by using different microwave coupling structures [108].

The radio frequency (RF) and microwave (MW) plasmas have some distinct advantages over the DC discharge plasmas. The advantages arise from the fact that the main electrodes or excitation source do not need to be located inside the vacuum chamber. Thus some common problems like electrode corrosion, gas contamination can be minimized.

Surface wave plasma (SWP) is an electromagnetic excitation that propagates in a wave-like fashion along the planar interface between plasma and a dielectric medium, and whose amplitude decays exponentially with increasing distance into each medium from the interface. Thus, SWPs excite a surface electromagnetic wave, whose electromagnetic field is confined to the near vicinity of the dielectric – plasma interface. A model structure for the interaction between the two interfaces is presented in Figure 25. The plasma is produced in the area below the dielectric medium it is cold and homogenous with electron density n_e, electron-neutral collision frequency $\vartheta$and relative permittivity

$$\varepsilon_p = 1 - \frac{\omega_p^2}{\omega(\omega + i\vartheta)}$$

where ω_p is the electron plasma frequency. The finite surface impedance of the metal walls is neglected and the only losses taken into account are the collision losses.

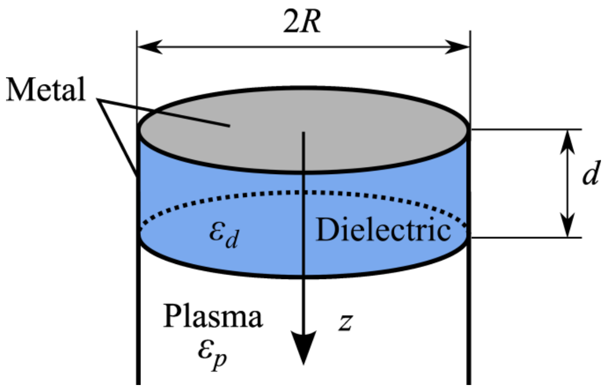


Figure 25. Surface wave propagation described by two-interface model [109]**.**

Depending on the condition used to obtain the plasma there are two distinct operation modes: surface wave plasma and volume wave plasma. If we are speaking about over - dense plasma, the electromagnetic wave is reflected from the plasma surface and becomes evanescent wave. On the other hand for under-dense plasma in addition to surface modes, volume waves modes can be obtained [109].

During our study we employed two similar surface-wave plasma devices. Both of them consist of a stainless steel chamber with diameter and heigth of 400 mm, respectively 600 mm as seen from Figure 26. Surface wave plasma is produced by launching 2.45 GHz microwaves through quartz windows via slot antennas. The discharge is excited using ammonia gas and mixtures of ammonia and argon as it will be discussed in results chapter. For the 400 mm chamber the total flow of gas mixture was kept constant at 100s ccm and the working pressure during plasma processing was 13 Pa. In the case of 600 mm chamber we used 150 sccm total gas flow and the experiments were performed at 5 Pa. The microwave power is feed into the chambers using waveguides equipped with E-H tuners, water-cooled isolators, directional couplers, and monitor coupler. The whole system is powered by a 2.45 GHz magnetron generator.

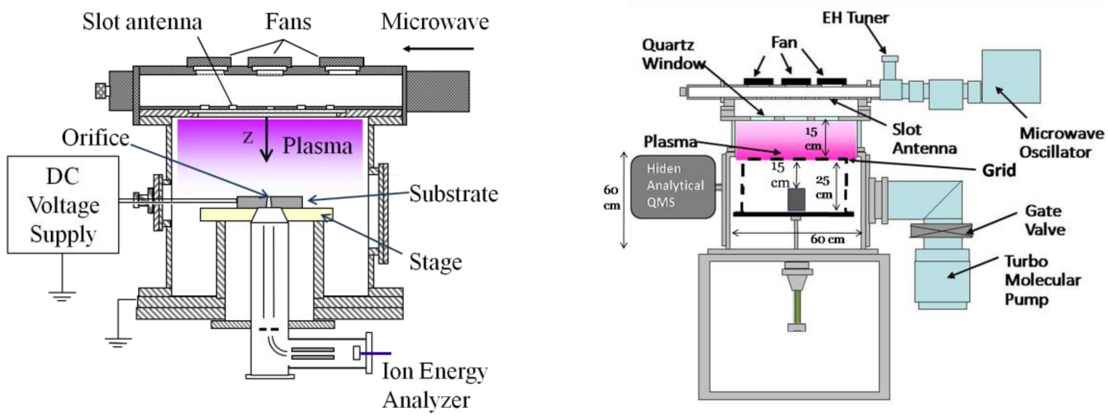


Figure 26. Schematic draw of Surface Waves Plasma devices:
400mm chamber (left), respectively 600mm chamber (right).

The above described experimental setups were both used during my study due to some particularities that offered the posibility to perform specific measurements. Due to the configuration of the devices it was possible to measure exclusively on the 600mm diameter vacuum chamber the influence of the species in plasma on zinc oxide materials by using a grounded grid between the plasma and sample that allowes only the neutrals to pass from the plasma side toward the samples. In the case of 400mm in diameter vacuum chamber the high performance plasma sampling mass spectromether enables the ion mapping under all plasma conditions. Detailed discussion is presented in the Results section.

4.2.3 Characterization of the surface wave plasma

In order to understand the plasma processing and optimize it, we have diagnosed it in different conditions. Two methods were used: optical emission spectroscopy, which gives details about plasma radiative species, and mass spectrometry, offering information on ionic, radical, neutral components and their enrgy profiles. Results are discussed in detaill in Chapter 4 and 5.

.

Plasma functionalization of zinc oxide nanostructures

5.1 Testing the possibility of adding amine groups on zinc oxide surface

5.1.1 Motivation

We started our study with the idea in mind to promote zinc oxide as suitable material for the development of new bioimaging application. As it can be seen from Figure 27, we propose a two step processing technique:

1. in the first step we plan to add amine groups on the surface of the zinc oxide by **plasma processing** and,
2. in the second step we want to used the functionalities as reactive covalent sites to connect specific ligands that can further connect different molecules, depending on the desired outcome.

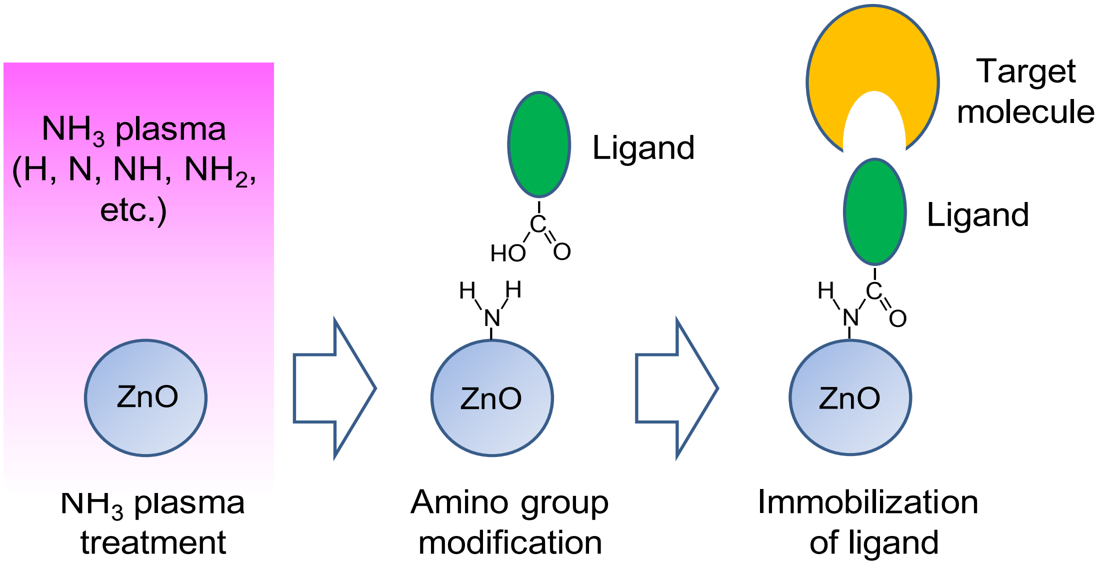


Figure 27. Illustration of biofunctionalization of zinc oxide nanoparticles and utilization in bioapplications [106]**.**

5.1.2 Experiment description

To confirm the possibility of zinc oxide amination by plasma processing, we first used commercial zinc oxide powder (Nilaco Co. P-15 #137 – low-pressure use) with micrometer size particles pasted on silicon wafers. In Figure 28 TEM image of the micrometer size commercial powder is shown.

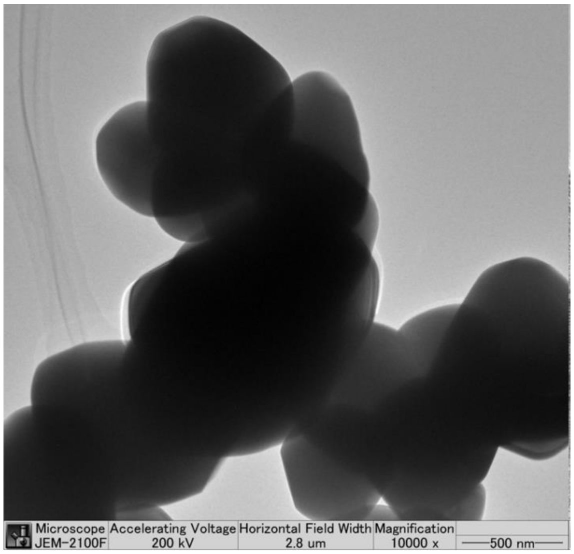


Figure 28. TEM image of commercial ZnO powder [106]**.**

OES measurement were preformed to prove the existence of a proper reactive medium for amine functionalization. We used the surface wave plasma (400mm chamber) to treat the zinc oxide using different conditions for the plasma discharge. The samples were positioned at 100 mm under the quartz plate.

Two parameters were changerd in order to compare different conditions. First the value of the microwave power used to produce surface wave plasma was changed between 700 and 1000W. Secondly, pulsed bias of -100V and -150V at 5 kHz was applied on a mesh electrode placed under the samples to stimulate the defects production on the surface of zinc oxide powder by intensified ion bombardment. All the results were compared to those of direct plasma processing without biasing.

The outcome of the plasma functionalization was analyzed with chemical and physiochemical methods. To investigate the surface elemental and chemical composition we carried out XPS measurements of the material before and after plasma treatment for all the conditions. In this sense, we analyzed the atomic nitrogen concentation modification that can occurs during plasma treatment.

5.1.3 Optical emission spectroscopy of the ammonia excited surface wave plasma

Optical emission spectroscopy (OES) is an important tool for fast and accurate elemental analysis of elements. Optical emission spectroscopy provides a non-evasive probe to investigate atoms, ions and molecules within plasma. It can provide information about properties, such as excited state, species densities, electron-atom, atom-atom and ion-atom collisional effects, energy distribution of species, charge transfer between plasma constituents, and electric and magnetic fields. To better understand the plasma processing, the investigation of the plasma chemistry is needed. The OEM measurements performed during this study help developing of a precise plasma reactor able to achieve proper surface modification of the zinc oxide nanostructures.

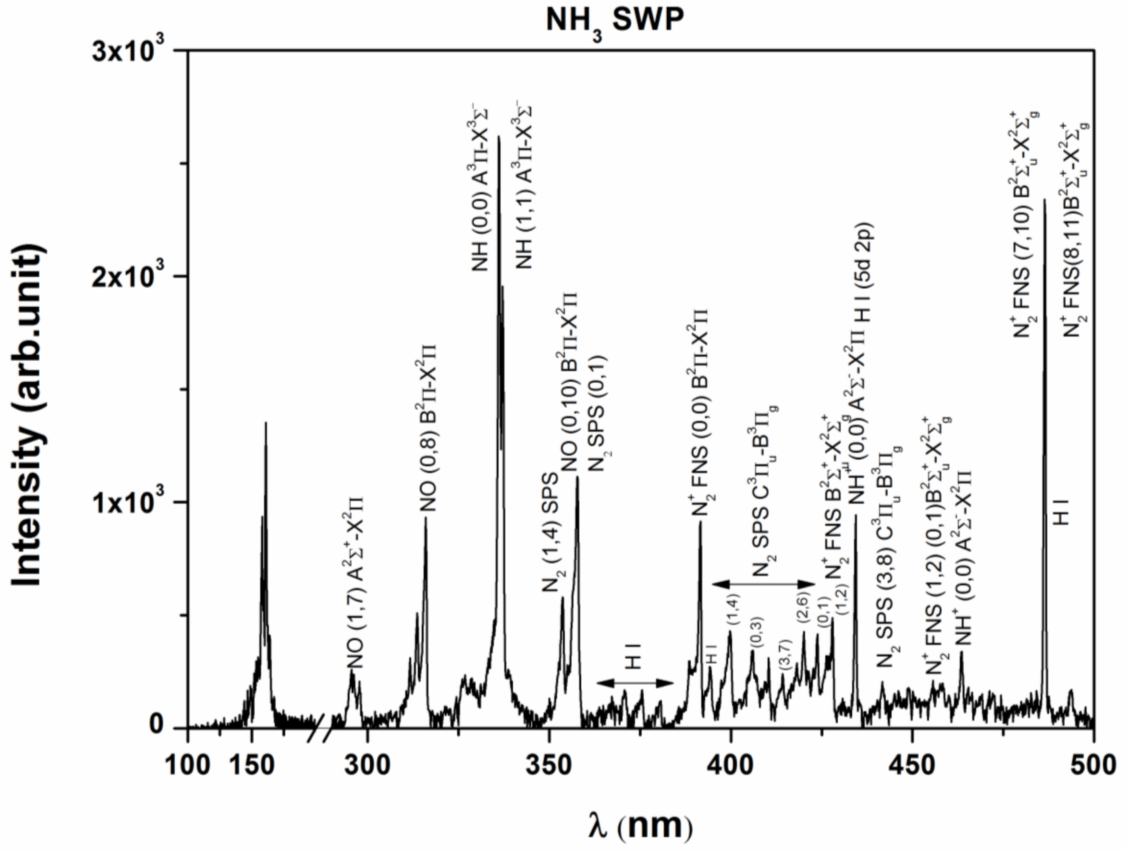


Figure 29. OES spectra of pure ammonia excites SWP [110].

The surface modification was performed in surface wave plasma excited in ammonia gas. In Figure 29 the typical OES spectrum in the UV-visible range of pure ammonia plasma is presented.

Ammonia excited SWP generates the necessary nitrogen containing species that could be responsible for sample amination: excited and ionic N, NH, NH_2, and NH_3 species, and possibly NH_4. The most significant NH radical line in our experimental conditions is the 336.1 nm line, which corresponds to the deexcitation of the NH_3. A NH radical can be created in plasma zone and other fractionation products (e.g. N, H) of the plasma might interact with the surface finally eliciting a surface-bound NH radical. The introduction of amine groups onto the surface of zinc oxide is mainly attributed to the existence of the active NH radicals. Other fraction products such as N, NH_2 also contribute to the introduction of amine groups. The second positive system of N_2 as well as the first negative system of the N_2 were also observed.

5.1.4 Results and discussion of ZnO commercial powder functionalization by plasma treatment

Figure 30(a) and (b) show the N 1s XPS spectra of the untreated zinc oxide micro-particles and the zinc oxide samples treated by ammonia SWP at 700 W, respectively. N 1s components are clearly enhanced in the case of ammonia SWP treated sample compared with the case of untreated samples.

N 1s peak can be deconvoluted in two components centered at 397.6 eV and 399.7 eV, corresponding to the secondary amines (=NH) and primary amines (-NH2), respectively, as shown in Figure 30(b). All the spectra of the processed samples have a similar deconvolution structure. ***Thus, the results indicate that plasma processing is able to add not only primary amines but also secondary amine groups as well on the surface of commercial micro-sized zinc oxide particles***. Considering the reactive species evidenced inside the plasma reactor, these results are reasonable and expectable.

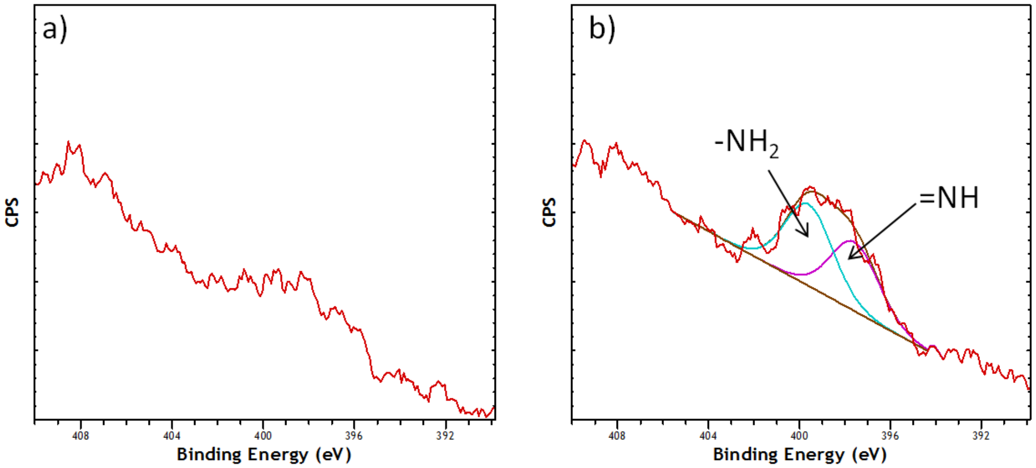


Figure 30. XPS spectra of N 1s for a) untreated ZnO particles and b) ZnO particles treated by ammonia SWP at 700 W [106]**.**

To examine the effect of ion bombardment on the surface modification, I compared the nitrogen content for three cases: no bias, biased at -100V, and -150 V, respectively. Figure 31 indicates that the highest nitrogen content for the same processing time, 5 min, was obtained in the case of the highest bias voltage, -150V. This shows that increasing ion energy favors the connections between the reactive functionalities and ZnO materials. So it is considered that reactive sites creation over the surface of ZnO particles due to the strong ion bombardment promote the subsequent reaction of functional moieties with the reactive sites. Comparing the results obtained for different biases and no bias, the higher nitrogen content detected (for the same processing time) was found for the highest bias voltage, -150V. This indicates that increasing ion energy the connections between the reactive functionalities and ZnO materials are favored.

For a further confirmation and quantification of the amine and amide functionalities, I have chosen a chemical method: ninhydrin assay, that was decribed in detail in Chapter 2. Ninhydrin is able to connect primary and secondary amine groups, the reactions releasing a purple (when reacting with primary amines) or yellow (when reacting with secondary amines) compound.

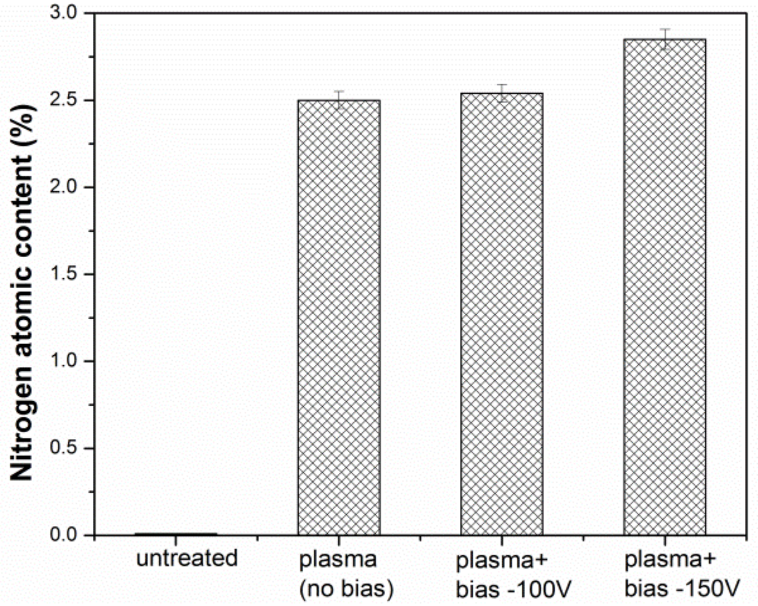


Figure 31. Nitrogen percentage determined from XPS N 1s spectra for untreated and ammonia SWP treated ZnO samples with biased substrate at -100 V, and -150 V for the same treatment time (5 min) [106]**.**

The quantification of the method is done using a spectrophotometer. According to ninhydrin assay, the absorbance determined with the spectrophotometer at 570 nm is proportional with the number of primary amine groups, while the absorption at 440 nm is proportional with the number of secondary amines in the sample.

Figure 32 clearly shows the evaluation for the direct plasma processing without bias as a function of processing time. According to these results on, both the number of primary and secondary amines in the samples increase with the increasing of processing time. There also seems to be a saturation of functional group addition after the first few minutes since the values determined for 5 and 10 minutes are not considerably different. All results determined for the SWP-processed ZnO samples show absorbance for both wavelengths in contrast with the untreated ZnO which doesn’t absorb for either values, indicating that in all studied cases successful functionalization of the ZnO materials by SWP processing.

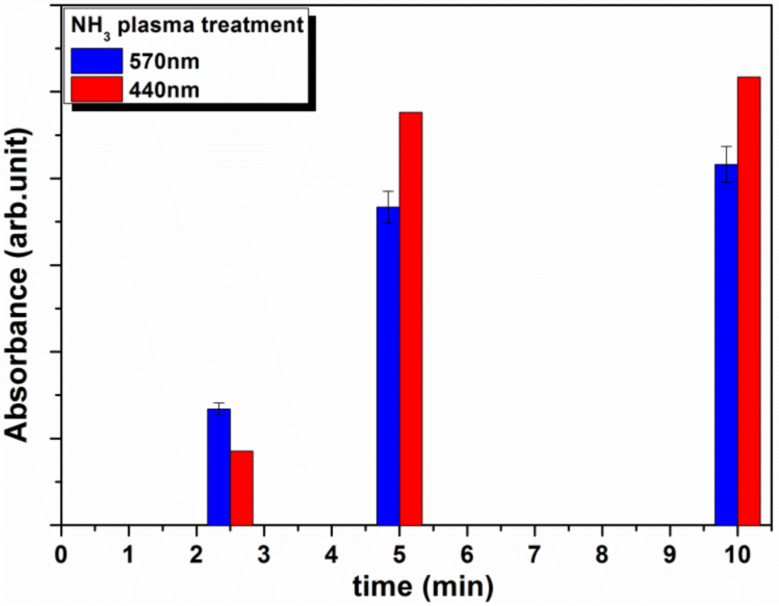


Figure 32. Ninhydrin assay results: sample absorbance as function of processing time [106]**.**

My preliminary results about the surface modification of zinc oxide by plasma processing offer the premises that the functionalization of zinc oxide is achievable trough this technique. We are the first to propose this novel approach on amine functionalization of zinc oxide materials by plasma processing.

5.2 Functionalization of zinc oxide nanoparticles

5.2.1 Motivation

As it has been already emphasized in the Intoduction of this thesis, it is essential to make use of nanomaterials when dealing with molecular level biological processes. Already having the premises that functionalization is achievable by ammonia excited surface wave plasma processing, in the next step of my work I focused on the use of zinc oxide nanoparticles obtained by pulsed laser ablation as raw material for our experiments. The motivation for this choice is mainly given by the optical properties, not to forget about the other mentioned advantages of ZnO.

5.2.2 Experimental considerations

The production of nanoparticles with controlable size and good crystalinity was alredy described in detail in the previous chapter. Thus, in this chapter I shall only emphasize on plasma surface modification of such nanoparticles.

We were able to obtain zinc oxide nanoparticle with controllable size of a few nanometers and crystalline structure as it can be seen from the TEM images in Figure 33.

By analizing TEM images with dedicated software (Olympus Soft Imaging Solutions – measureIT) , we estimated the size of the nanoparticles showing that our technique offers the posibility to produce nanoparticles with diameters less than 20 nm, as it can be seen from the distribution plot in Figure 34.

As previously stated, our interest focuses on improving the properties of zinc oxide nanomaterials by plasma functionalization and thus the obtained materials by laser ablation will serve as support for some our experiments.

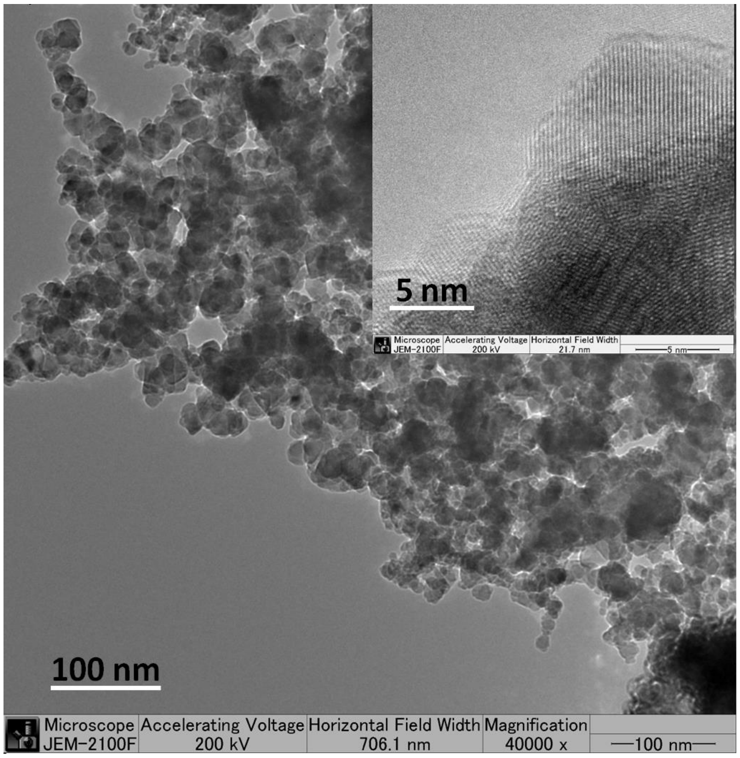


Figure 33. TEM image of zinc oxide nanoparticles fabricated by pulsed laser ablation [106]**.**

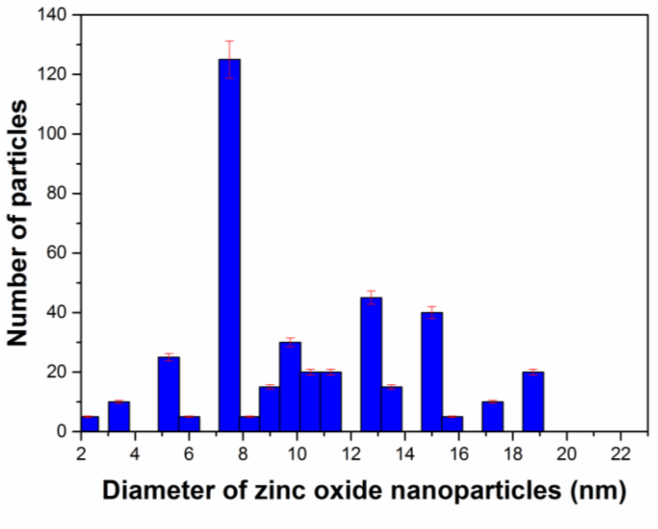


Figure 34. Size distribution of zinc oxide nanoparticles prepared by laser ablation.

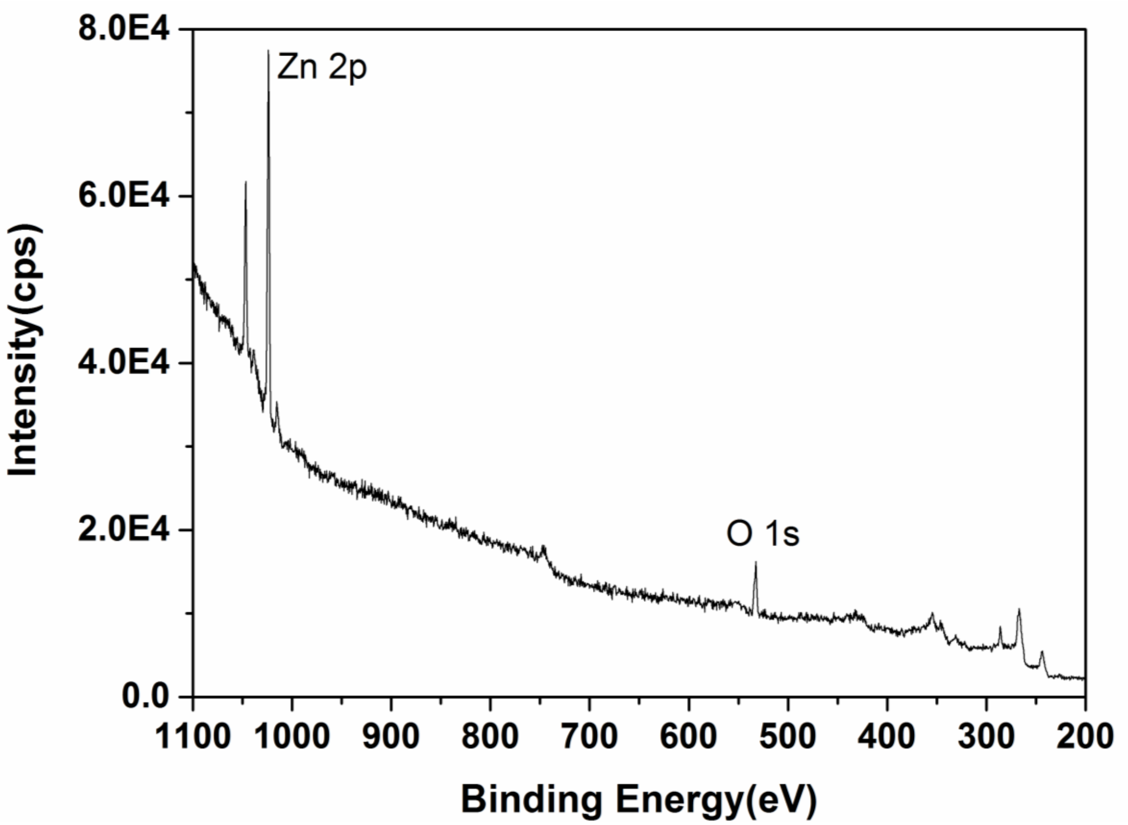


Figure 35. XPS wide spectra of PLD obtained zinc oxide nanoparticles.

As expected the XPS measurements of the as obtained zinc oxide nanoparticles with the pulsed laser deposition technique show the typical pristine zinc oxide spectra as seen from the Figure 35.

In this new stage of my work I performed similar experiments as in the case of micrometer size zinc oxide commercial powder, trying to find the best functionalization parameter to be able to optimise the amine functionalization of our samples. In the same time, I try to understand which plasma species, neutrals or ions, have a role in plasma functionalization.

5.2.3 Results and discussions

I employed once again the XPS analysis and, as it can be seen from Figure 36, the nitrogen content is increasing for the processed sample in surface wave plasma ignited for grid power of 700 W and by biasing the substrate with a -100 V 5 kHz pulsed voltage. The results are in good corelation with those determined for the micrometer size powder.

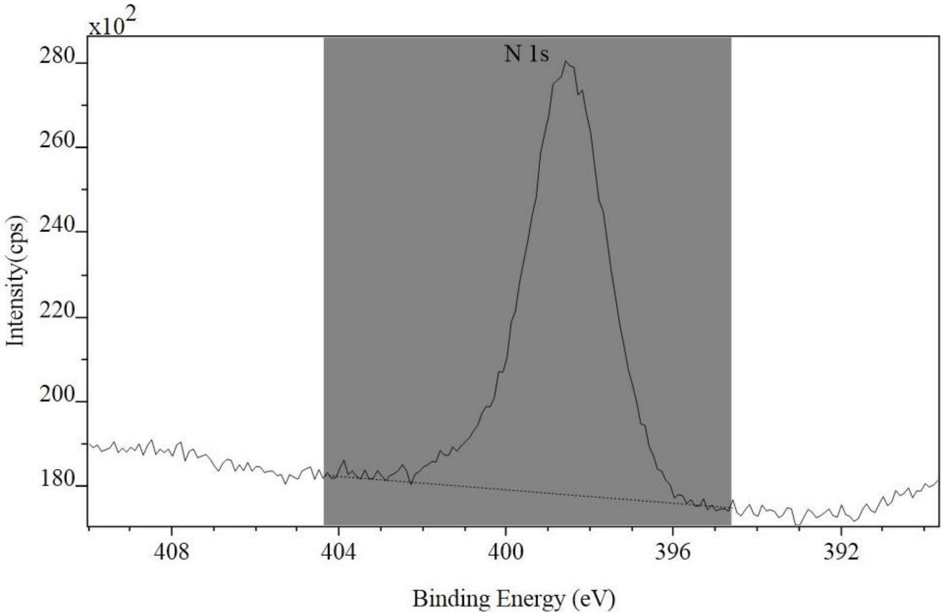


Figure 36 XPS spectrum of N 1s for ZnO nanoparticles treated by ammonia SWP at 700 W with -100 V biased substrate [106]**.**

After proving by XPS the succesful introduction af amine groups on the surface of nanoparticles, I used a complementary method to check functionalities by connecting them to a fluorescent dye and then imaging the sample at the fluorescent microscope. As a fluorescent dye I used Alexa Fluor 488 that specifically connects amine groups . The measuring principle is schematically shown in Figure 37.

Figure 38 shows the images of a) pristine zinc oxide nanoparticles that were not exposed to ammonia SWP, and b) zinc oxide nanoparticles processed in ammonia SWP, both after reaction with fluorescent dye. It is clearly seen that in the first case there is no fluorescence so there are no amine groups, while the green light in the second case proves the presence of amine groups on the surface of zinc oxide sample.

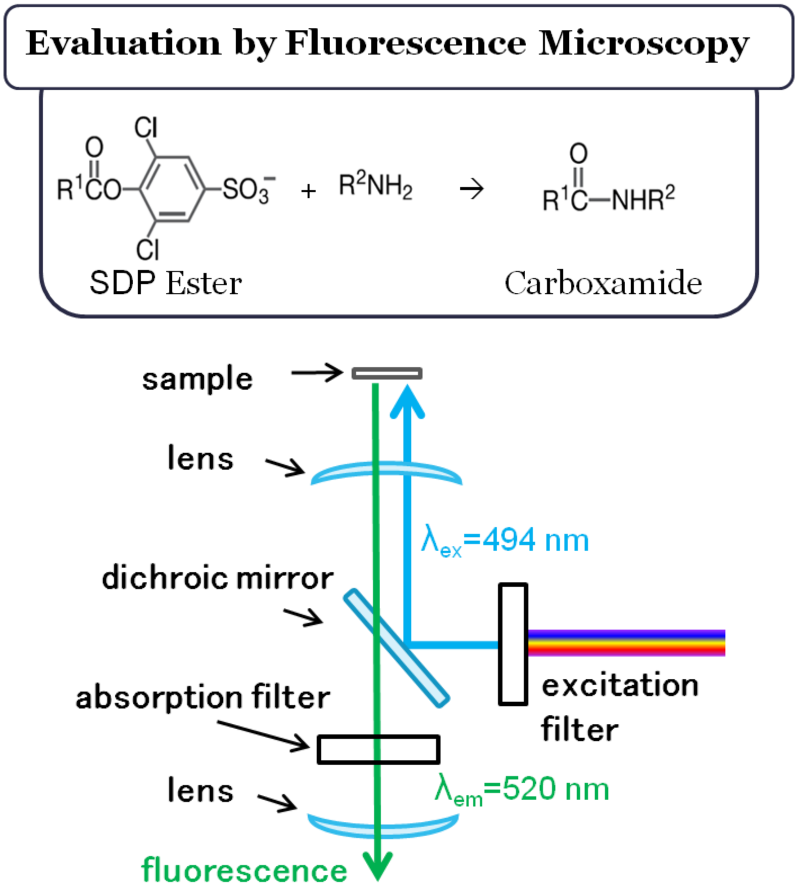


Figure 37. Schematic diagram of the fluorescent microscope.

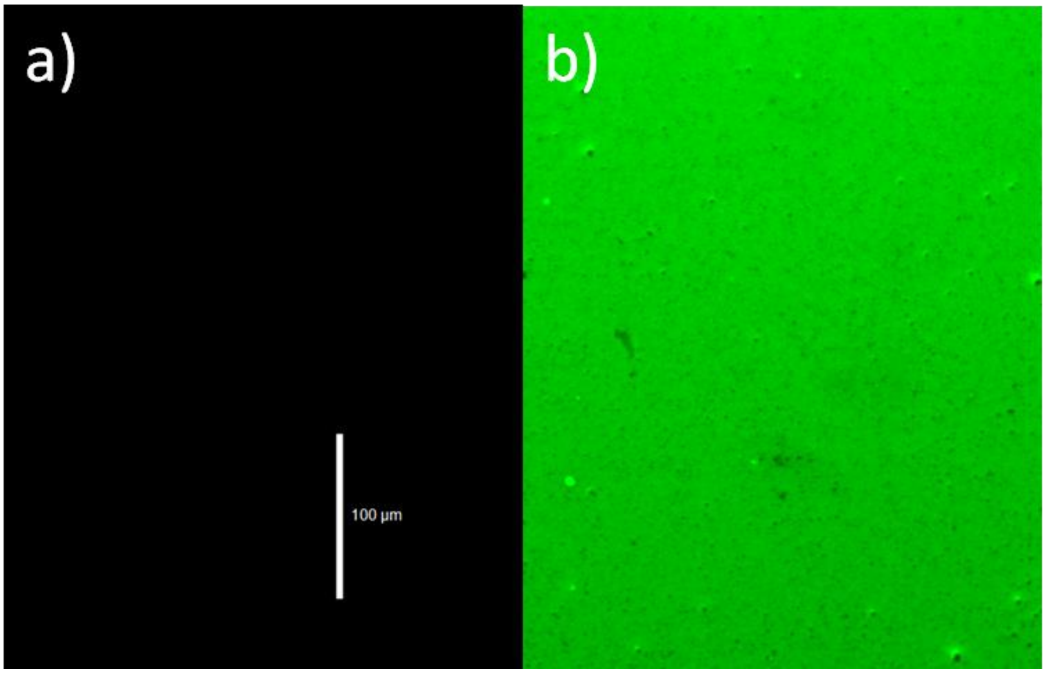


Figure 38. Fluorescent microscope images: a) untreated, and b) SWP processed zinc oxide nanomaterials [106]**.**

The fluorescent microscopy results and the XPS measurements confirm the success of this study and its applicability to nanostructured zinc oxide.

Already identifying from the OES measurements the presence of NH radical, I started to investigate in detail which other contribution from plasma could contribute to the addition of amine groups on the surface of the nanomaterials. To know exactly which plasma species are responsible for the introduction of the amino functionalities and to distinguish between the ionic and neutral component, I had conducted a new study in which 2 batches of samples were prepared: one was exposed directly to the plasma and for the other one most of the ions were stopped by a grounded grid placed between plasma and the samples.

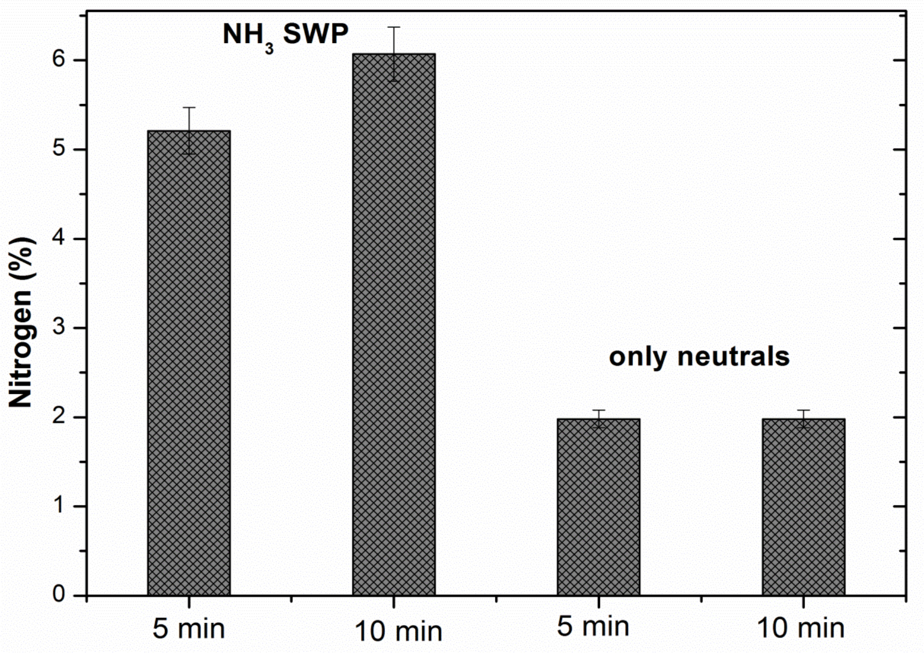


Figure 39. Influence of the species from plasma reactor regarding the nitrogen content introduction on the samples.

The XPS analysis presented in Figure 39 clearly shows that the nitrogen atomic concentration is enhanced, probably due to the presence of the energetic ions in plasma and only to some small extent to the neutral species since only under neutral treatment the number of amines introduced is considerably smaller. Samples were exposed to plasma for 5 minutes and 10 minutes, respectively. These results have a valuable meaning, and correlated with the fact that functionalization of the samples can be enhanced by biasing the substrate during the processing, provide valuable insights of the plasma functionalization.

The point of the study is not only to provide a new approach on zinc oxide functionalization by adding functional amine groups, but also to check how the existing properties of the material are changing during the processing. It is know that pristine zinc oxide has a low dispersion in aqueous media and since our aim is to use these particles in biological media, most of the time aqueous, I also checked the dispersion properties before and after plasma processing.

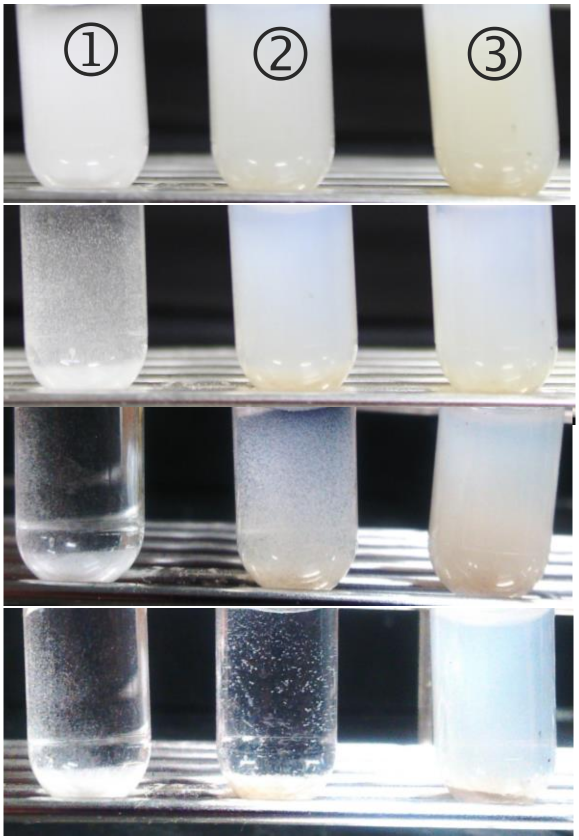


Figure 40. Comparative dispersion properties of: 1) ZnO commercial powder, 2) nanostructured ZnO, 3) functionalized nanostructured ZnO.

In Figure 40 a comparison is made between the dispersion of the zinc oxide commercial powder that we use in our initial study, the zinc oxide nanoparticles produced by laser ablation, and the plasma processed zinc oxide laser ablation nanoparticles. It is clearly seen that the zinc oxide obtained by PLD has a better dispersion in pure water that the commercial powder and even more, the dispersion of the plasma processed nanoparticles is much improved compared to the original one. From these results the conclusion is that plasma processing is a suitable method for adding amine groups on the surface of the zinc oxide and, in the same time, to enhance their dispersion properties.

Plasma is a reliable dry chemical reactor that can provide the necessary means to functionalize zinc oxide nano and micro size materials and to enhance the dispersion properties in aqueous medium. Unfortunately these processing methods need optimization for maximizing the outcome and, not to forget, for minimizing the cost of production.

Even if these results are encouraging, there is some critical issue to address: the possibility to use the bias voltage as to enhance even more the interactions between ionic plasma moieties and the samples to enhance the functionalization. This shall be discussed in next part of my work.

Optimization of the plasma processing technique for amine group functionalization of ZnO materials

6.1 Optimization of plasma parameters

6.1.1 Motivation

In the previous sections I have proved the benefits of plasma processing for micro and nano size structures functionalization and also discussed the influence of different plasma species for the introduction of amine groups on the surface of zinc oxide. In the last years different methods proved reliable and suitable for enhancing the energetic species in plasma. A good way to increase the effectiveness of the plasma processing and also to have stable plasma discharging conditions is achieved by feeding argon gas in the discharge.

Since the cross-section for the three body collisions expected to enhance the production of plasma ions depends on the percentage of argon and ammonia in the mixture used to ignite plasma, I investigated different mixtures where argon and ammonia were introduced in different proportions. Not only the functionalization is evaluated but also the properties of the ZnO materials.

6.1.2 Results and discussion

For this part of the study I used the 600 mm chamber. I first started by adding only small amount of argon in the mixture (10%) to see if the functionalization process can be improved. This time a comparison between pure ammonia plasma processing and mixture of 10% argon and 90% ammonia was made.

A very important aspect regarding zinc oxide and its optical properties arises from the defect presence in the lattice. To better understand the way amine groups connect to the surface of the zinc oxide, I've chosen another approach analyzing what happens with the oxygen vacancies in the lattice after plasma processing. In this sense in Figure 41 are presented spectra of oxygen O 1s for the as obtained zinc oxide from laser ablation (referred here as control) and for a sample processed in pure ammonia SWP and 1:9 Ar:NH_3 SWP.

By deconvolution, the O 1s peak is fitted with two peaks: one centered at 531.3 eV (blue), representative for the fully oxidized oxygen atoms, and a second one centered (yellow) at 533.5 eV, corresponding to the oxygen vacancies in the zinc oxide lattice. It is clearly seen that pure ammonia processing increases the number of defects in the lattice of the zinc oxide, a trend seen for both 5 minutes and 10 min processing time.

On the other hand, the mixture of 10% Ar and 90% ammonia has a different outcome showing a decrease of the number of vacancies in the zinc oxide lattice. This behavior can be related with the fact that argon supports the enhancement of the functionalization - required moieties in plasma. Knowing that the photoluminescence properties of zinc oxide nanostructures arise from the presence of the defects in the lattice, these measurements helped to better describe how the material behaves after plasma processing.

PL measurements were carried out for the ZnO samples treated in pure NH_3 SWP and also for those processed in the Ar:NH_3 gas mixture to understand the how the addition of the amino groups on the surface of the nanoparticles influences the photoluminescence properties of the zinc oxide. In Figure 42 a comparison of PL spectra for untreated zinc oxide and pure ammonia processing SWP for 1.5 minutes and 3 minutes with and without bias voltage is presented. An increase of the green emission intensity centered at 560 nm appears only for the samples treated for a short period of time (1.5 min). For longer treatment time (3 min) the green emission intensity of the sample is decreasing and the intensity of the sharp peak center at 391 nm is increasing. This can result from the way the amine groups are bound to the surface of the zinc oxide nanoparticles occupying the oxygen vacancies.

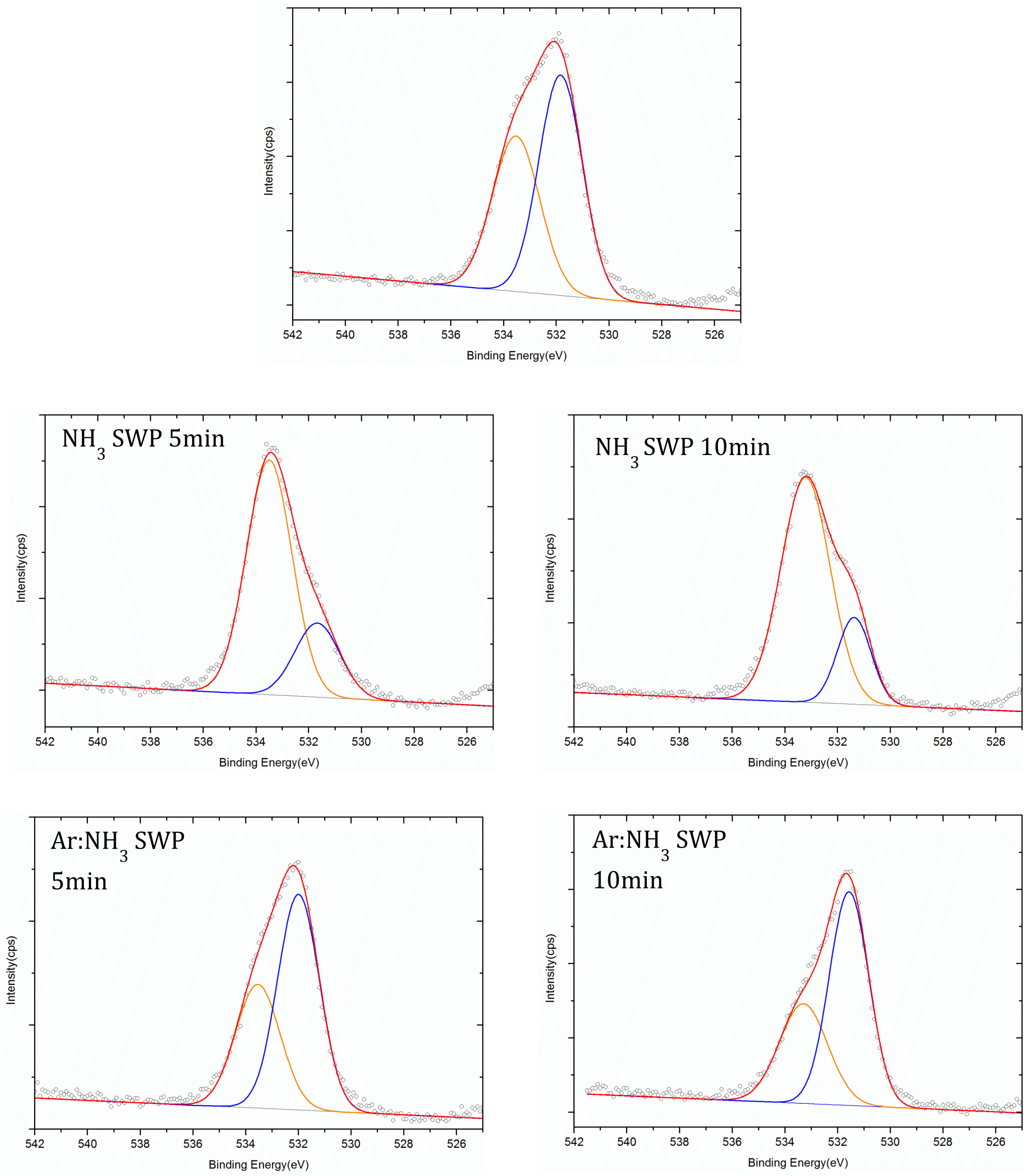


Figure 41. O1s spectra of unprocessed zinc oxide, pure ammonia SWP, and Ar:NH$_3$ 1:9 SWP treatments.

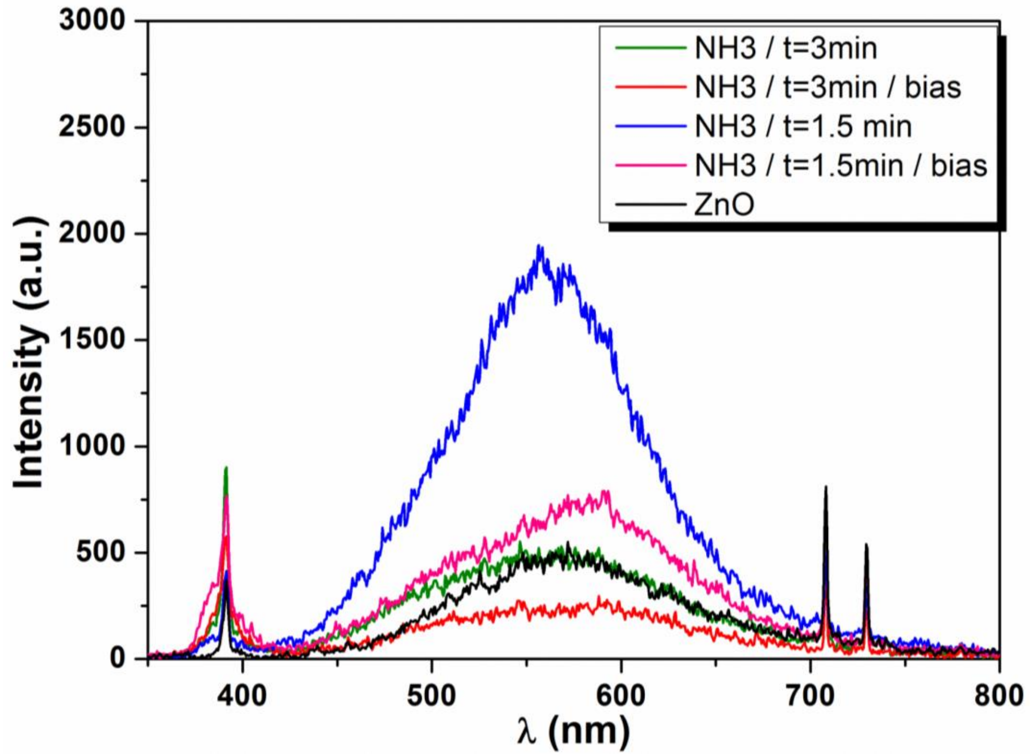


Figure 42. Photoluminescence spectra of processed zinc oxide in pure ammonia SWP.

In Figure 43 a comparison of PL spectra for untreated zinc oxide and a mixture of Ar and NH_3 processed in SWP for 1.5 minutes and 3 minutes with and without bias voltage is presented. Samples processed in SWP with a mixture of 10% NH3 with 90% Ar show an enhancement of the green band emission due to the stronger ion bombardment in the latter case and also longer treatment time proved a higher enhancement. Even if the use of bias voltage proved to facilitate the introduction of amine groups on the surface of zinc nanoparticles, there is no obvious enhancement of the green band emission in PL measurements.

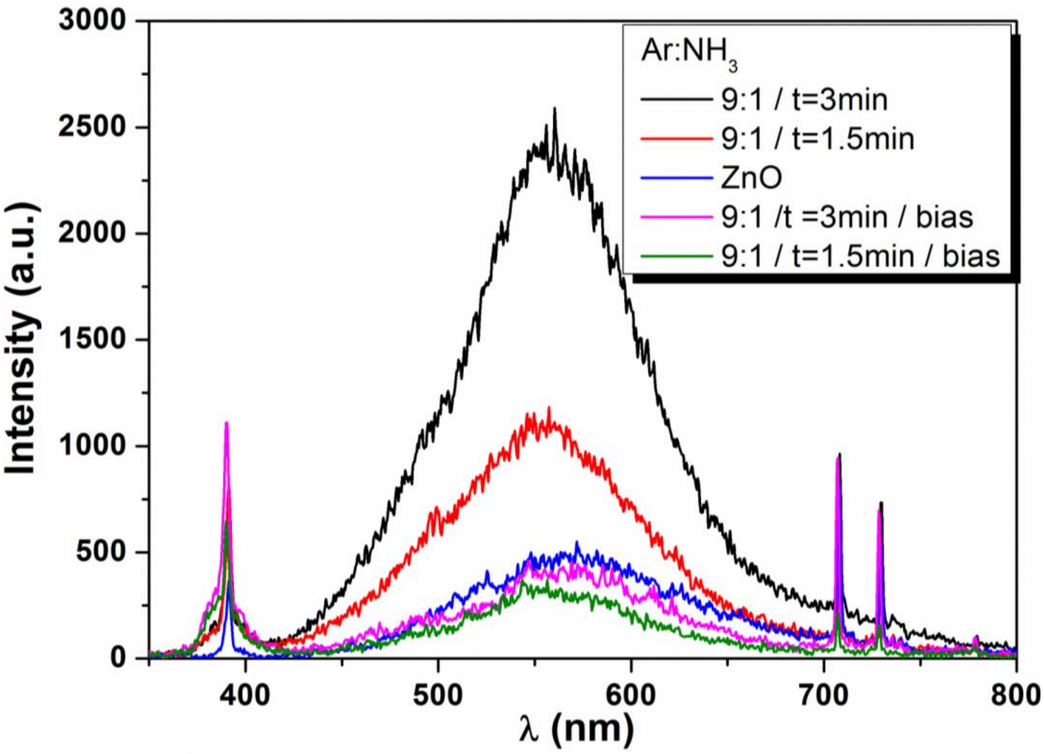


Figure 43. Photoluminescence spectra of processed zinc oxide in Ar:NH$_3$ 1:9 SWP.

To optimize the functionalization process, I have studied in detail how different ratios of argon, starting from 10% to 90%, in mixture with ammonia influence the plasma treatment of the nanomaterials.

As seen from the photoluminescence measurements the emission spectra in the visible range is represented by a broad peak and thus the emission it cannot be attributed to only the oxygen vacancies in the lattice. In this sense I deconvoluted the emission peak as it can be seen from Figure 44. Deconvoluting the peak, it seems that this visible emission is primary due to the oxygen vacancies (V_O) in the zinc oxide lattice (green emission) and to a small extent to blue and yellow emmision contributions due to the interstitial zinc (Zn_i) in the lattice.

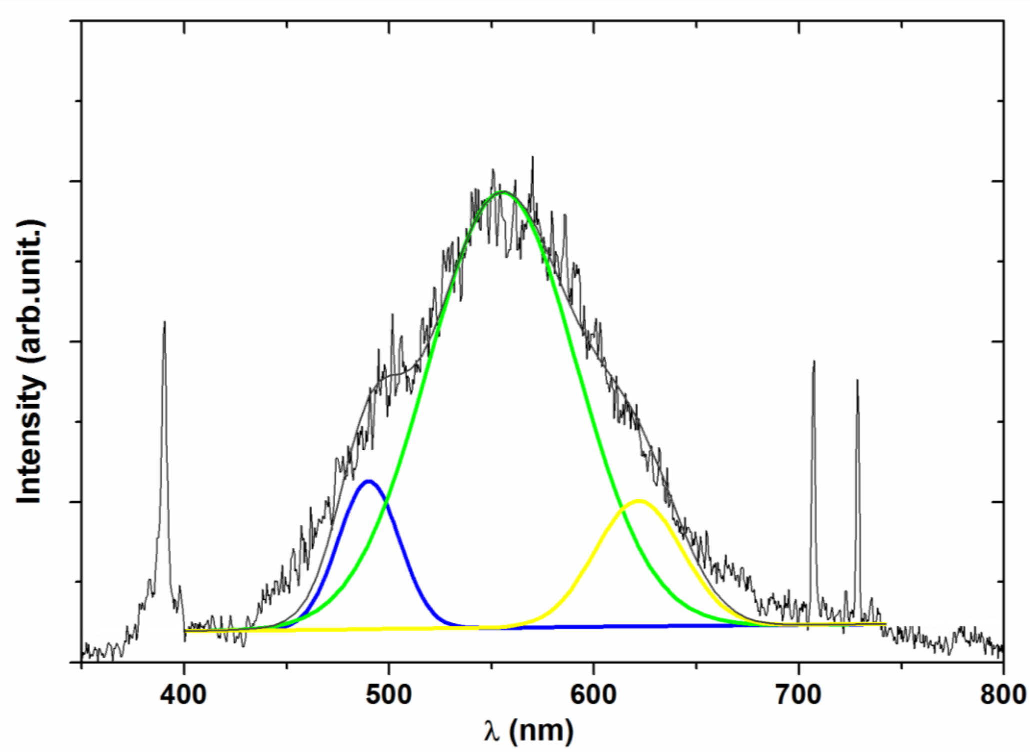


Figure 44. Deconvolution of the photoluminescence peak of the zinc oxide.

6.1.3 Optical emission spectroscopy of the argon and ammonia gas mixture excited surface wave plasmas

Previous studies regarding the modification and addition of functional groups on the surface of different nanostructures materials revealed an enhancement of some of the species present in pure ammonia plasma when argon was introduced in the discharge [6], [84], [111], [112].

It is expected that the presence of argon metastables will provide a higher ionic density and that argon ions will bombard the surface of nanoparticles, facilitating further interaction of functionalization moieties (e.g., NH, and NH_2 ionic or neutral components) with the samples. To map the plasma under different NH_3/Ar gas mixture conditions, the emission lines of the species excited in NH_3/Ar SWP shown in Figure 45 were studied.

The analysis of the spectra clearly shows the emission peak for NH at 336.1 nm among the second positive system (SPS) of N_2 and the first negative system (FNS) of N_2^+ at 391.4 nm, which are related to the species in the plasma responsible for the functionalization of zinc oxide nanoparticles with amine groups. Compared with the case when only pure ammonia gas was used to excite the surface wave plasma when adding argon in to the discharge the emmision peaks for the moieties responsible for the functionalization are enhanced.

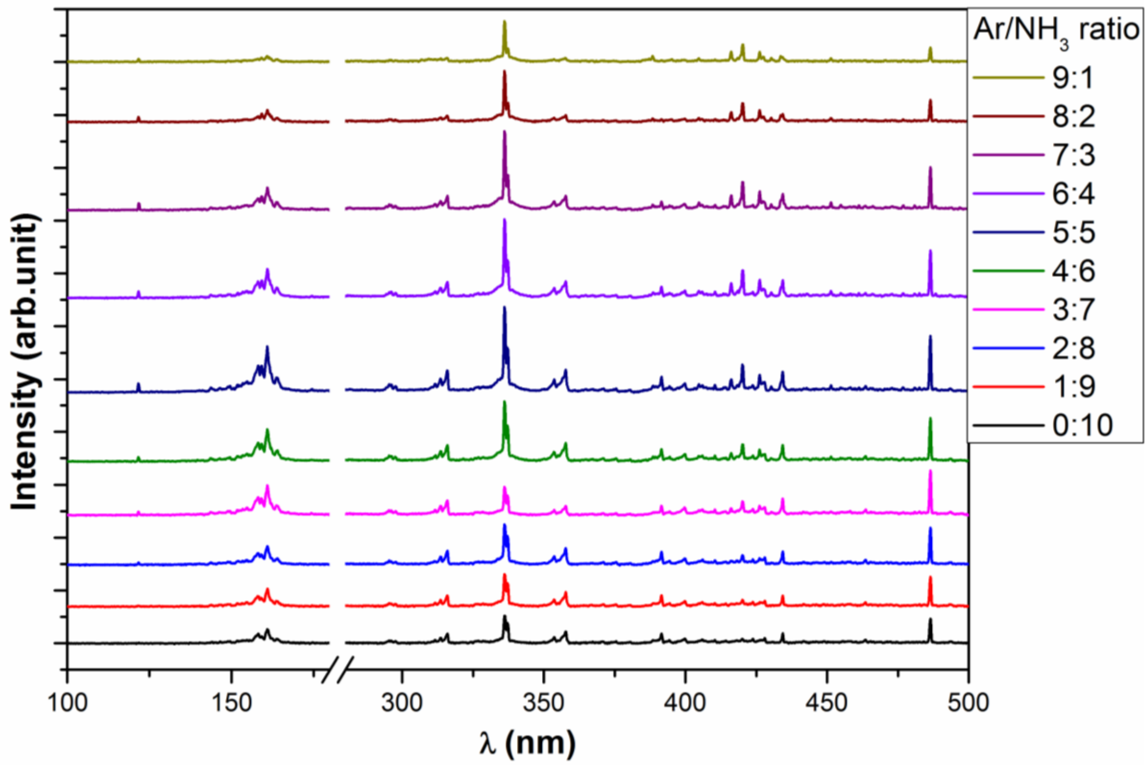


Figure 45. OES spectra comparison for different mixtures of Ar and NH_3 used to excite SWP [110]**.**

Another important aspect is represented by the atomic H_β (486.1 nm), which results from the fragmentation of the NH_3 molecules, and it's responsible for the creation of NH_2 species, and also for the process of etching, that might occur during sample processing. The latter is competitive to functionalization. A plot of these two is shown in Figure 46. It should be considered that the balance between functionalization and etching is essential in determining the ideal parameters of the process. The highest intensity of NH and H_β was attained for NH_3/Ar mixtures ratios ranging from 30% NH_3/70% Ar to 60% NH_3/40% Ar.

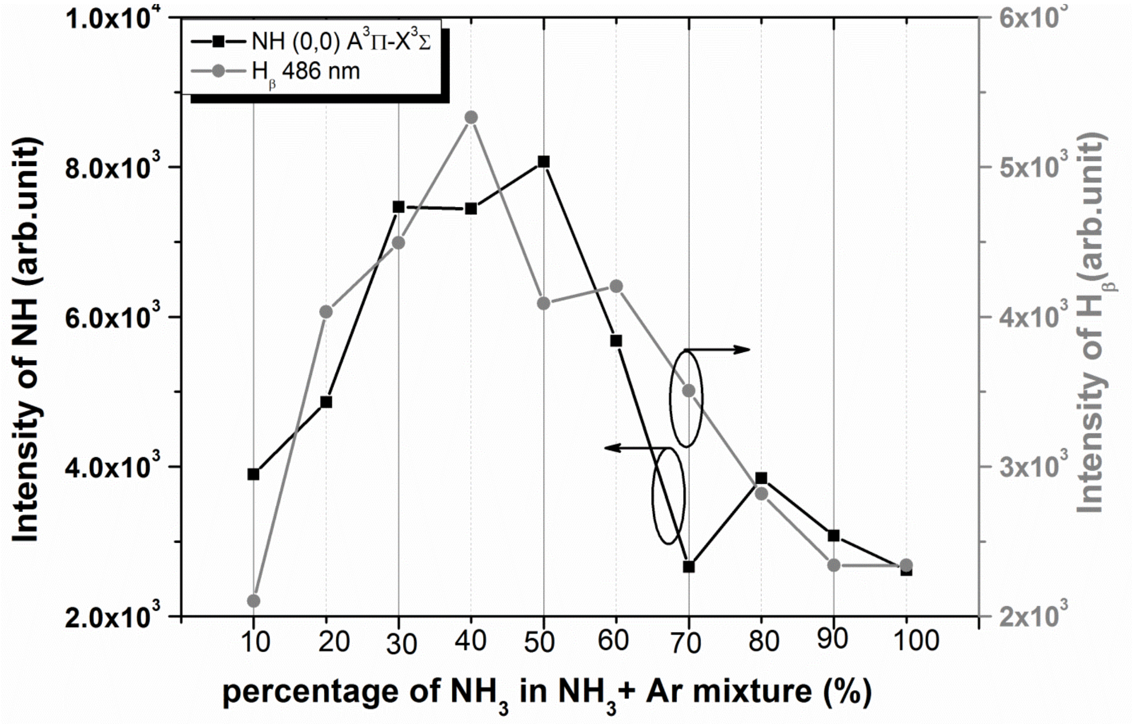


Figure 46. Comparative plotting of the emission intensity of the NH and H_β lines for different mixtures of NH_3/Ar used to generate the SWP [110].

6.1.4 High-performance plasma sammpling mass spectrometry

The quadrupole mass filter is a widely used mass analyzer that has benefited from 30 years of continual commercial development and refinement. It consists of four accurately aligned parallel metal rods that are arranged symmetrically around a central axis that is the path of ion movement from ion source to ion detector. An electrical field is created around the central axis by DC and radio frequency potentials placed on the rods, with opposite rods connected together. The electrical signals placed on the rods determine the paths that low-kineticenergy ions follow through the rod structure. At a given DC potential and RF frequency, only ions within a certain mass range follow a stable ion trajectory that allows them to reach the detector rather than collide with the rods themselves. The oscillating field applied to the rods alternately attracts and repels ions passing through the mass filter, inducing an ion motion that is exploited to differentiate ions on the basis of their mass [113].

To understand the machanism of plasma fucntionalization of zinc oxide nanostructures we used a Hidden Analitycal PMS003 high resolution quadrupole mass spectrometer. High-performance plasma sampling mass spectrometry measurements enable the ion mapping under all plasma conditions, in terms of species, and their densities and energy profiles.

Figure 47 shows the densities of the ions assumed to be responsible for the functionalization in pure ammonia and 10% NH_3/90% Ar gas mixture SWP. By correlating these results with the OES measurements, it was found that the most important ions responsible for the enrichment of zinc oxide nanoparticles with amine functionalities might be NH^+, NH_2^+, NH_3^+, and N_2^+. As expected, the presence of Ar in the mixture facilitates the dissociation of NH_3, and an enhancement of the formation of the H^+ and H_2^+ ions arises.

A comparative study of NH^+, NH_2^+ , and NH_3^+ for different gas mixtures used to generate SWP is presented in Figure 48. It is clearly observed that, for NH_3^+, the density is one order higher than that for NH_2^+, and two orders higher than that for NH^+. In the ion profiles, we can also observe the presence of H_2O^+ (18 amu) and NO^+ (30 amu) due to the water vapor and residual air existing in the system prior to plasma ignition.

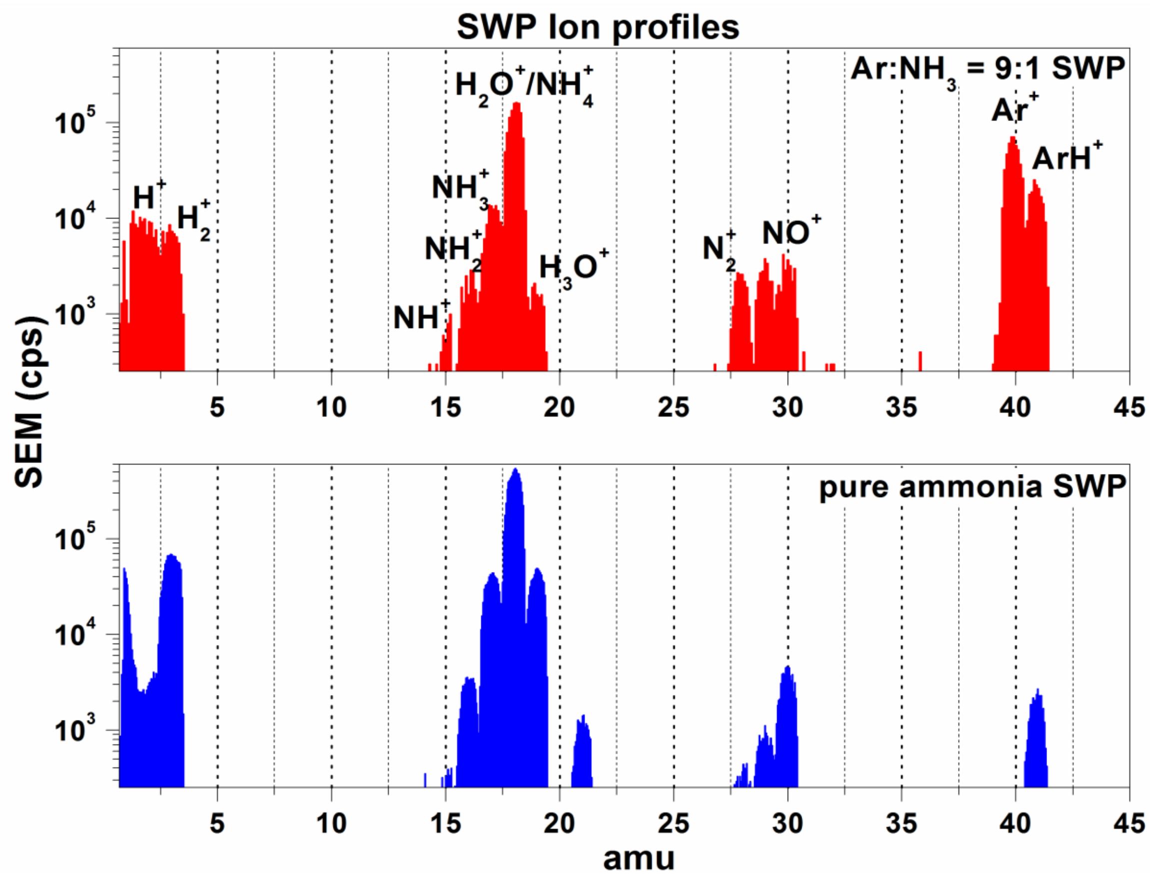


Figure 47. Comparative plotting of ion profiles in pure NH_3 SWP and mixture of 10% NH_3 and 90% Ar SWP [110]**.**

The presence of N_2^+ in the QMS measurements directly correlates with the OES results, which indicated the presence of the SPS of N_2 and the FNS of N_2^+. The same behavior of ion density is found for the ionic energy profiles of the studied mixtures, similarly for all the ions of interest (Figure 49).

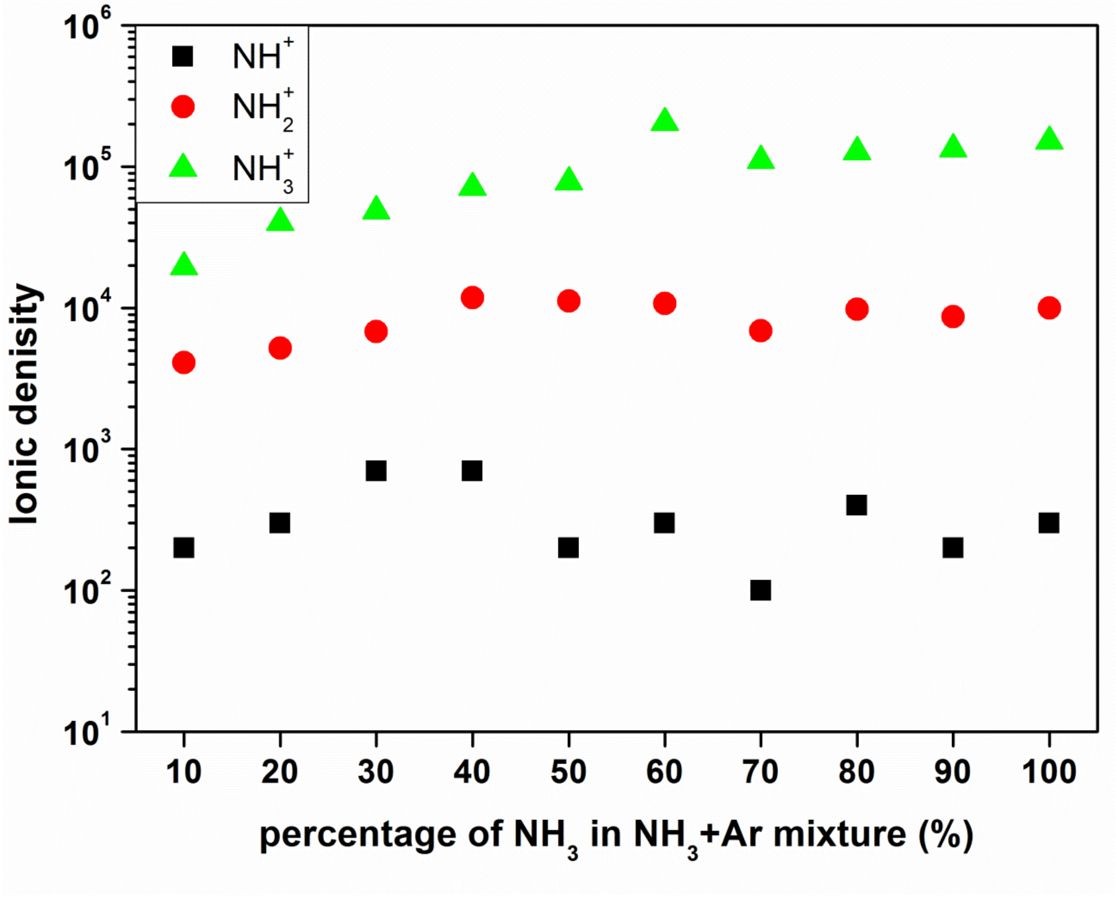


Figure 48. Ionic densities of the NH^+, NH_2^+, NH_3^+ different mixtures of NH_3/Ar used to generate the SWP [110]**.**

The energy profile is considerably smaller for high than for low ammonia content of the discharge gas mixture (all spectra are represented on the same scale). Moreover, in the case of 30% NH_3/ 70% Ar, NH_2 ions seem to have the highest energy. The energy profiles for the other ions are similar. From the ion density distribution under all studied conditions (Figure 48) as well as the ion energy distributions, we can deduce that for 30% NH_3/ 70% Ar, the ion component of plasma has the highest density and energy.

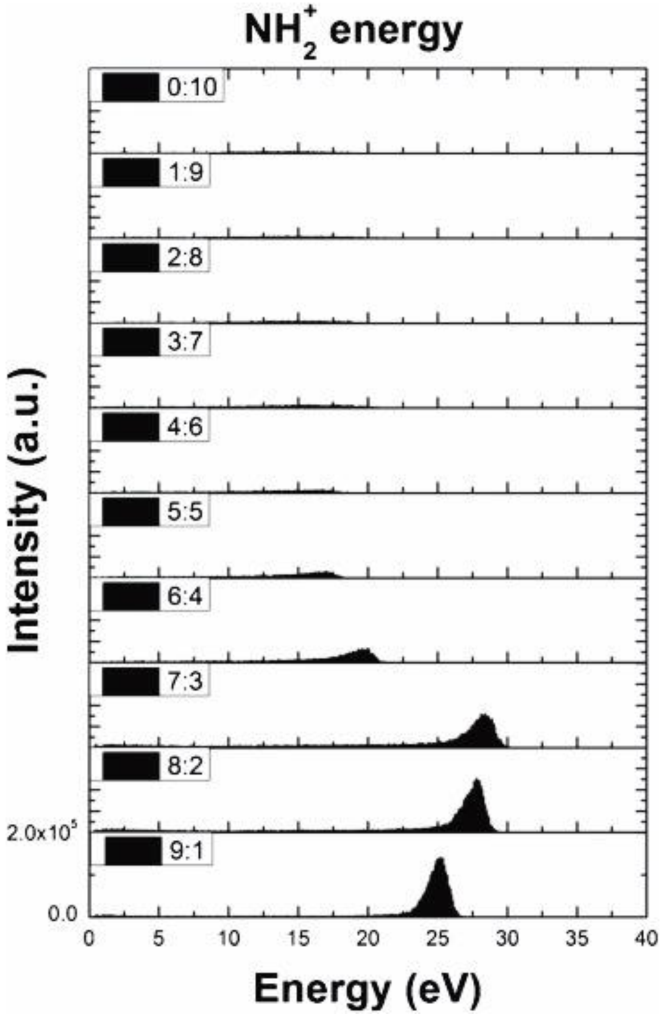


Figure 49. Ionic energy profiles of different mixtures of NH_3/Ar [110]**.**

Since the ion acceleration experiments indicated that ions have a major role in functionalization and considering the above-mentioned results, we can assume that the mixture of 30% NH_3/ 70% Ar would be better than pure ammonia SWP for amine group introduction into zinc oxide nanoparticles.

6.1.5 Outcome of plasma functionalization using mixtures of argon and ammonia

OES and QMS measurements give a clear idea of the processes taking place in the plasma reactor. Previously it was showed that there are two competitive processes that occur in our plasma processing: one is the functionalization of the surface of zinc oxide due to the proper moieties present in the discharge, and the second is the etching effect occurring due to the hydrogen species created from the dissociation of the ammonia molecules. All of this been said, the best processing parameters is given by a mixture of argon and ammonia ranging from 30-40% NH_3 and 70-60% Ar.

This conclusion is sustained by XPS measurements for a full set of samples treated for all mixtures of gases as it can be seen from Figure 50. From these results it's clear that the functionalization of the zinc oxide by low temperature plasma processing can be further enhanced using mixtures of argon and ammonia to excite the plasma with no further need of the biased substrate.

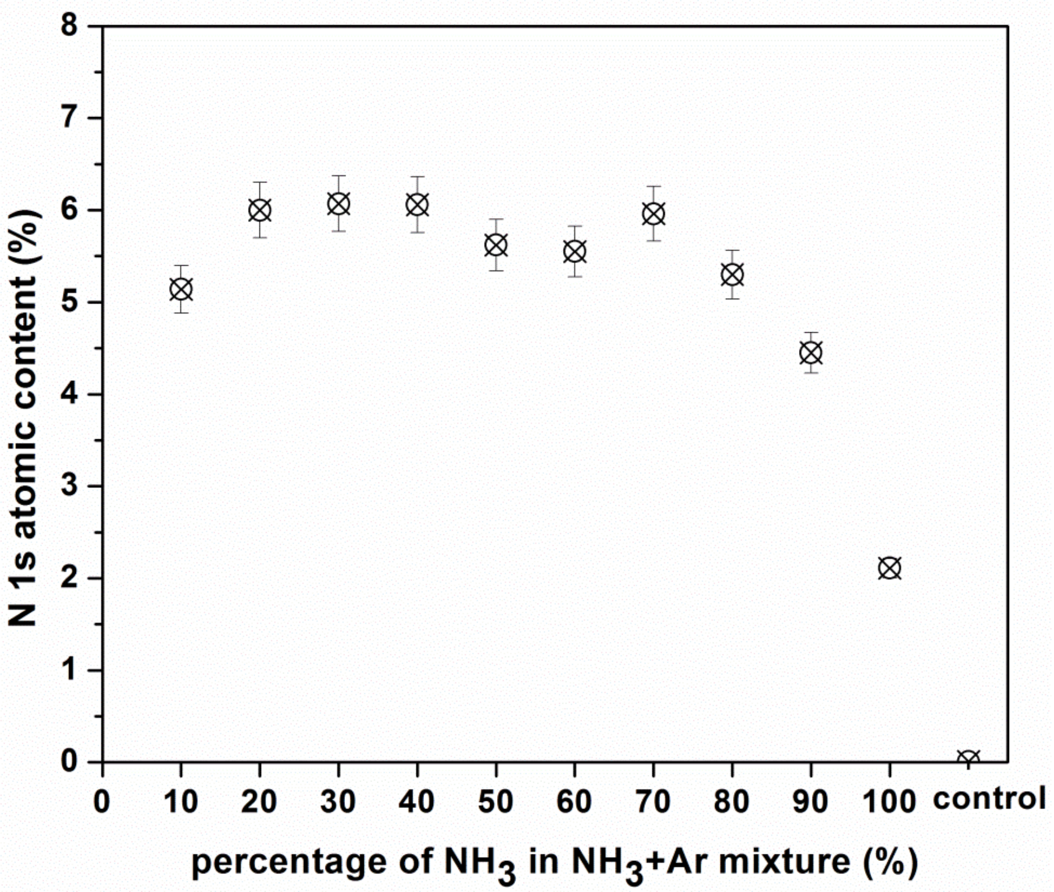


Figure 50. N 1s atomic content of ZnO nanoparticle samples in diffrent Ar/NH_3 mixtures compared with ZnO as obtained by PLD [110]**.**

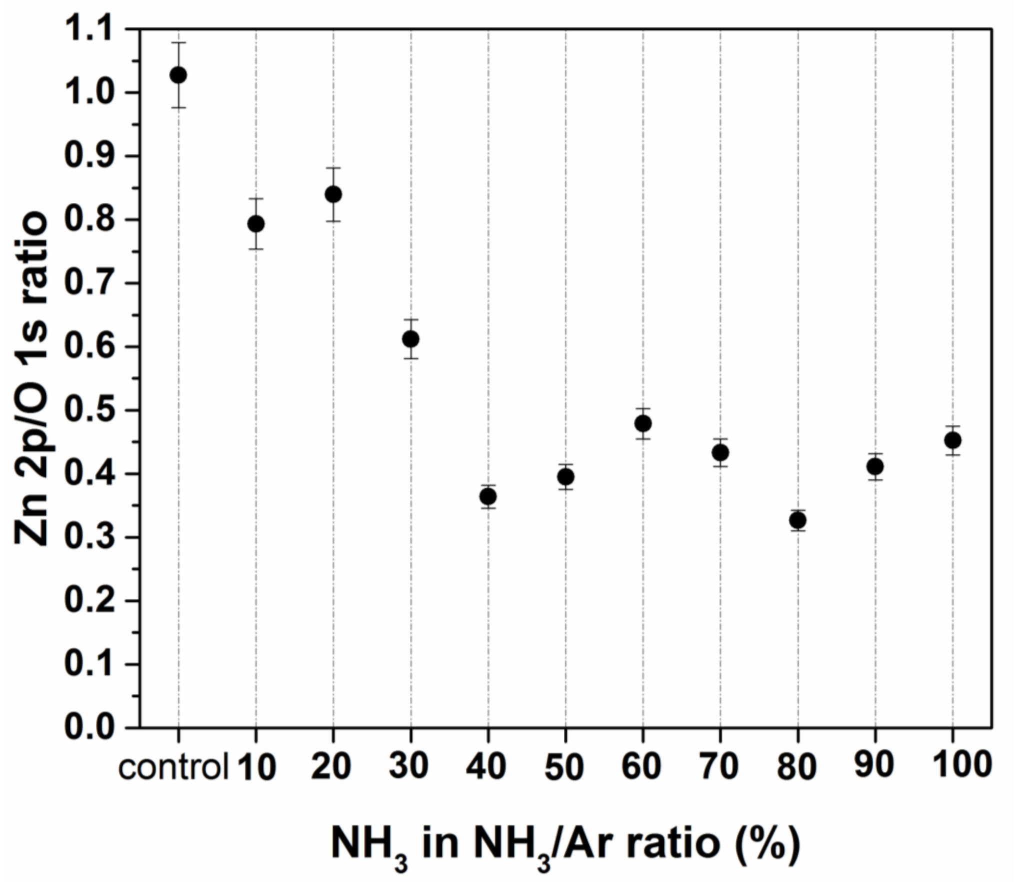


Figure 51. Zn 2p/ O 1s ratio for samples processed in different mixtures of NH_3/Ar.

In the same time, the photoluminescence of the zinc oxide can be enhanced for nanostructures when mixtures of gases are used, mainly due to the strong bombardment of the lattice by the argon ions.

Analyzing the XPS spectrums of the processed samples in different mixtures of NH_3 and Ar gases the ratio of the Zn 2p and O 1s can be calculated as seen from Figure 51. In the ideal case for the perfect zinc oxide the ratio is equal with one. As seen from the XPS measurements the pristine zinc oxide has the ratio of zinc and oxygen atoms of approximately 1. Despite this after plasma processing it is shown that this ratio is decreasing with the increase of ammonia percentage in the mixture. This could be due to the creations of new defects during plasma treatment, like oxygen and zinc vacancies. Even if it is known that the functionalization outcome is enhanced when mixture of NH_3/Ar is used, and mainly for high concentrations of Ar in the mixture, we cannot show how the addition of the amine groups on the surface of the materials is done and complementary methods are needed in this sense.

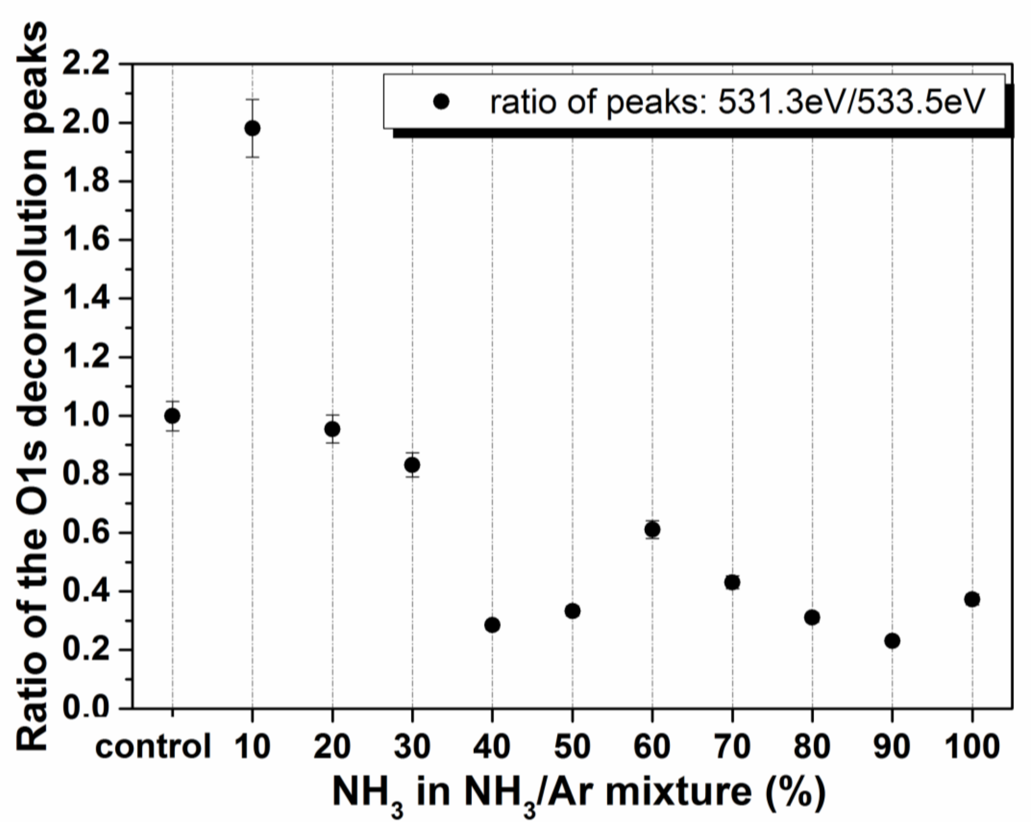


Figure 52. Deconvoluted O 1s peaks ratio for samples processed in different mixtures of NH_3/Ar.

As shown previously, the O 1s peak from the XPS measurements can be deconvoluted with 2 peaks centered at 531.3 eV and 533.5 eV and thus the ratio of fully oxidized atoms and oxygen vacancy can be calculated as seen from Figure 52. For pristine zinc oxide this ratio is approximately 1 and it is clearly seen that after processing this is changing depending on the mixture used to excite the surface wave plasma. For pure ammonia plasma the ratio shows a decreased compared with the original one suggesting that the number of defects in the lattice of the zinc oxide (oxygen vacancies) is increasing. For the cases when mixtures of argon and ammonia are used the ratios of fully oxidized atoms and oxygen vacancies are smaller than for the untreated zinc oxide but with the increase of the argon percentage, and mainly for the values for which the maximum functionalization with amine groups can be achieved, the ratio is almost the same like in the case of untreated zinc oxide. The highest value of the ratio, even bigger than the original zinc oxide, is in the case of 10% Ar and 90% NH_3 suggesting that the number of oxygen vacancies is highly reduced.

6.2 Quantification of the number of amine groups by chemical derivatization

To quantify the reactive sites, represented by the amine groups, a chemical derivatization method is employed. The method was described in detail in the previous chapter. Here only discussions about the results and the methods used to calculate the approximate number of bonded amine are presented in detail.

For the quantification of the amino groups bound on the surface of the zinc oxide nanoparticles I used Sulfo-LC-SPDP (sulfosuccinimidyl 6-[3(2-pyridyldithio)propionamido]hexanoate) protocol, from Thermo Scientific. Following the reactions in Figure 23, the absorbance (given by the Pyridine 2-thione molecules) at 343 nm is measured with the spectrophotometer. By calculating the difference in absorbance between the treated samples and control it is possible with the help of Beer-Lambert law to calculate the molar concentration.

This value is used to approximate the number of amine groups per microgram of substance. Figure 53 shows the concentration of pyridine 2-thione molecules measured from the absorbance at 343 nm that is equal with the number of amine groups introduced on the surface of the zinc oxide by plasma processing.

The values calculated in the same way for the nanopowder are presented in Figure 54. As it can be seen from the plot of amine groups number versus the percentage of argon and ammonia in the mixture used to excited the surface wave plasma, the maximum is obtained for the 30% NH_3 and 70% Ar. Once again these results are in strong correlation with the previous ones.

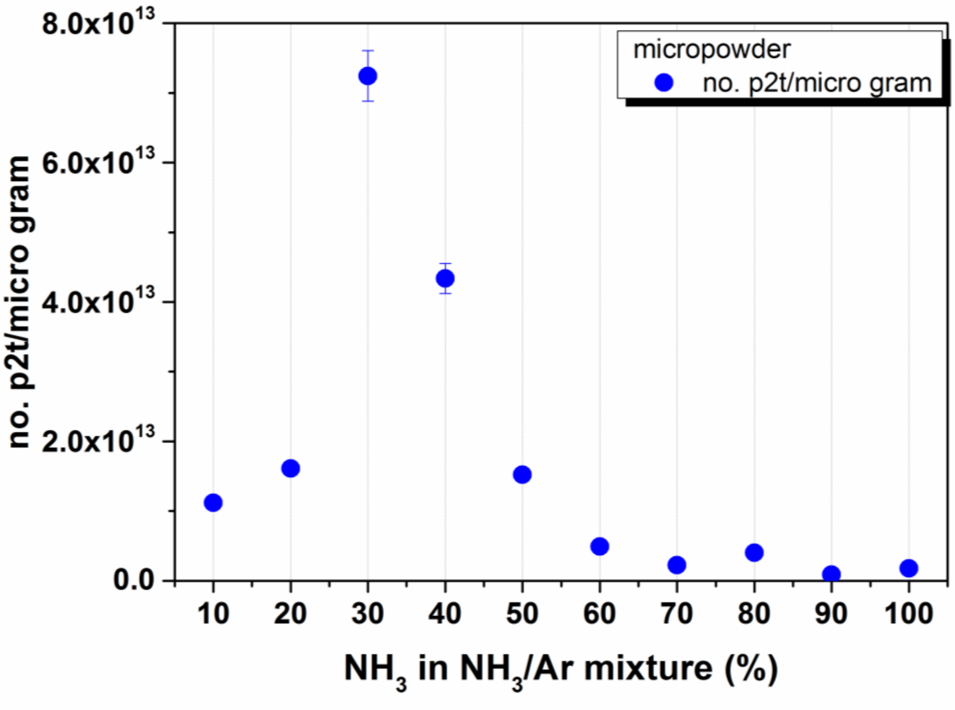


Figure 53. Quantification of the number of amine groups per microgram introduced on the surface of micropowder size ZnO by plasma processing.

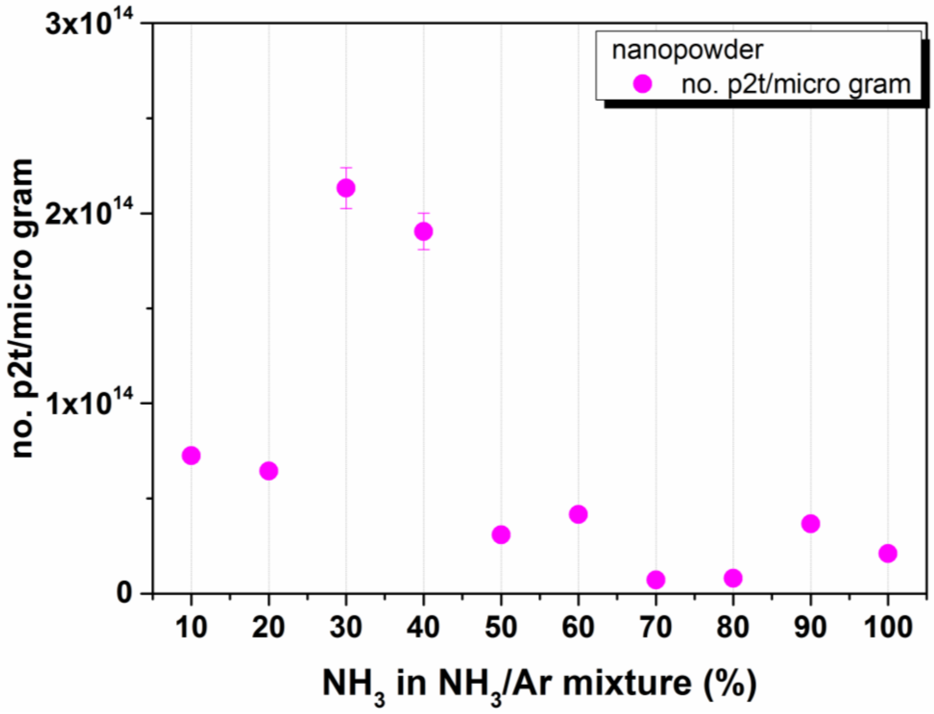


Figure 54. Quantification of the number of amine groups per microgram introduced on the surface of nanopowder ZnO by plasma processing.

6.3 Biofunctionalization of zinc oxide with biomolecules

After the optimization of the plasma functionalization process I proceeded to the next step of this study: analyze the possibility of connecting biomolecules with the plasma functionalized zinc oxide materials. As a biomolecule, a sugar chain was used: dextran. Sugar chains can serve as spacing molecules, having the ability of further binding biomolecules, offering biocompatibility properties and higher stability. A fragment of fluorescent dye labeled dextran molecule is schematically represented in Figure 55.

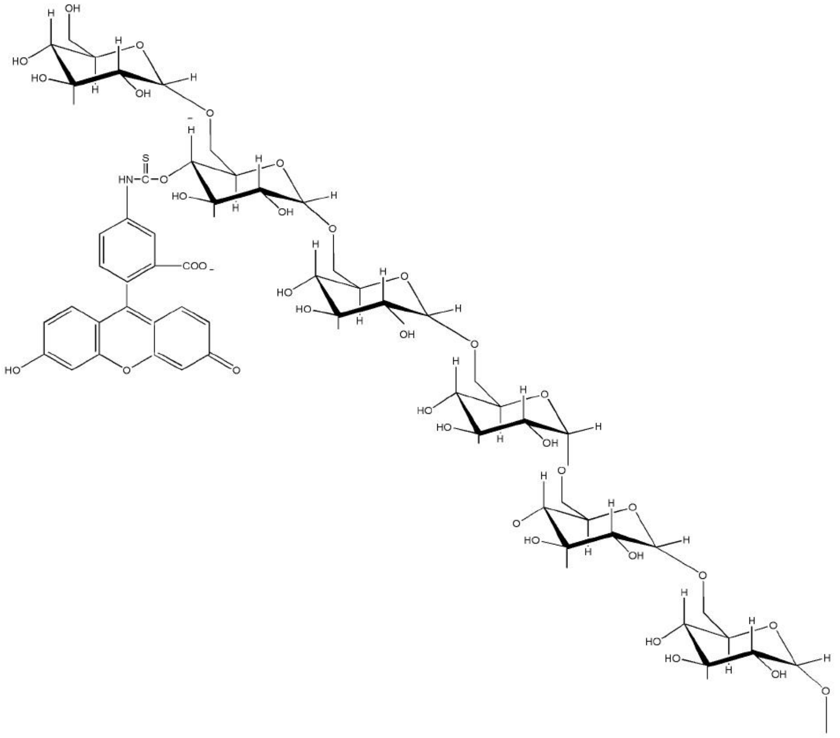


Figure 55. Structural representation of fragment of FITC-dextran molecule.

To check the connection between dextran and aminated zinc oxide two types of dextran were used: fully oxidized dextran and already labeled with fluorescent de dextran, FITC-dextran. In the case of oxidized dextran is expected that it will connect with the amine groups on the surface of functionalized zinc oxide. After removing the excess of dextran by several washing procedures with pure water, a label molecules (6-DTAF) is used to connect with the loose hydroxyl groups of dextran. The outcome of this reaction can be analyzed by fluorescence microscopy. When exciting with 494 nm wavelength it is possible to measure the fluorescence at 518 nm as it can be seen from Figure 56. Visually the control sample, which was not subjected to plasma treatment, does not connect with labeled dextran and thus no fluorescence can be observed. In comparison the plasma processed zinc oxide strongly connects with the dextran as seen from right picture in Figure 56.

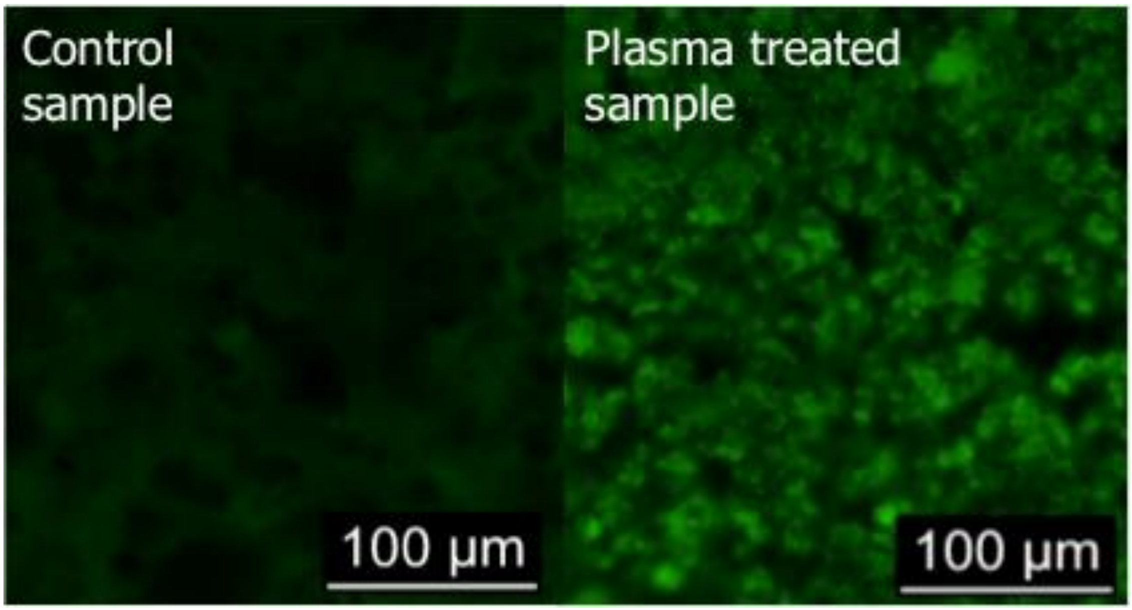


Figure 56. Fluorescence microscopy: (left) unprocessed sample and (right) plasma processed zinc oxide connected with oxidized dextran.

In the second case I used fluorescent dye labeled dextran and I repeated the process as previous described. As it can be seen from Figure 66, the unprocessed zinc oxide cannot connect with the dextran and thus no fluorescence can be observed. Despite that, plasma treated ZnO connects the biomolecules as it can be seen from right picture in Figure 57. The unprocessed sample still shows some weak fluorescence emission but that is only due to some dextran that adhered to the surface of the material, porous as it was due to particulate surface structure, and could not be removed by washing.

These result confirm the possibility to connect biomolecules by the newly plasma created reactive sites represented by amine groups on the surface of the zinc oxide materials. New opportunities arise regarding the development of specific targeting and imaging applications.

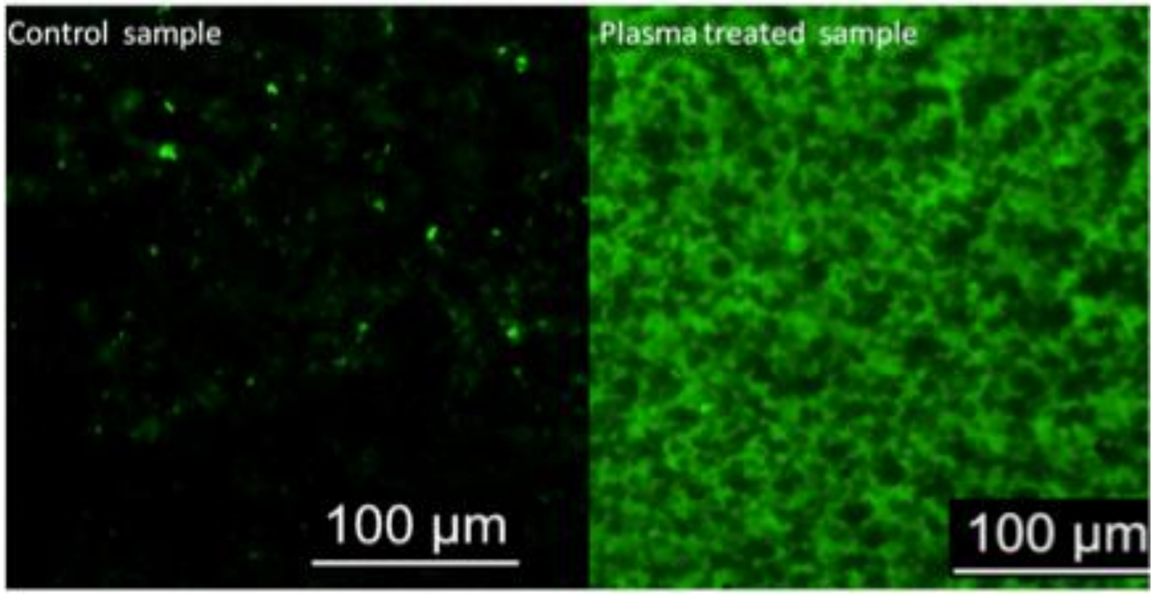


Figure 57. Fluorescence microscopy: (left) unprocessed sample and (right) plasma processed zinc oxide connected with labeled FITC-dextran.

6.4 Conclusions

In this chapter I analyzed in detailed the possibility to functionalize the zinc oxide materials by plasma technologies. I was able to provide valuable insights about the functionalization process and its optimization. First of all, I proved that plasma processing is a viable and simple alternative to the wet chemical methods widely employed. I provided information about the functionalization of the zinc oxide and described the most suitable conditions to enhance the functionalization process.

Surface wave plasma is produced in different mixtures of ammonia and argon and is used to functionalize the zinc oxide nanomaterials. To discuss the mechanisms, we used optical emission spectroscopy and high-resolution plasma sampling measurements. These two techniques offer important information on the creation of reactive species in the plasma that are involved in the functionalization of zinc oxide nanoparticles. The ions in the plasma seem to play a very important role in the processing. We were able to enhance the density of plasma by adding argon in the discharge gas mixture. Argon facilitates the dissociation of ammonia and the production of ionic species, responsible for the introduction of amine groups. Because of the presence of H^+ ions, we observed another process-plasma treatment that involves two competing effects: the creation of NH_2 radicals, which are directly related to the introduction of the amine groups, and the creation of hydrogen radicals, which act mainly as the etchant.

Regarding the optical properties of zinc oxide, I've proven the possibility of enhancing the photoluminescence of the materials after plasma processing. This is an important step towards the development of detection methods making use of the remarkable optical properties of zinc oxide.

By chemical derivatization I quantified the number of amine groups introduced on the surface of the plasma processed zinc oxide materials. Last but not least, I shown the possibility to connect biomolecules to the zinc oxide by making use of the amine groups introduced on the surface by plasma processing.

Conclusions

In this thesis I propose a novel approach on functionalization of the zinc oxide by employing for the first time the dry plasma processing of this kind of material. In order to develop bioimaging techniques that can offer better insights of the biological processes, I propose and prove that is possible to enrich the surface or zinc oxide with amine groups by plasma processing. I successfully functionalized different types of particles ranging from commercially available nano and micro powder to lab-made PLD nanostructures with controllable size and shape.

Through this thesis I provide information not only about the functionalization process itself but also about the optimization of the plasma parameters in order to achieve the best results. Optical and physical properties of processed zinc oxide were presented in clarifying the plasma benefit over the wet chemical methods widely used for functionalization of the materials.

To summarize the study, the following key points are offered throughout the thesis:

- ✧ **I promote the use of low temperature surface wave plasma processing as an efficient method for functionalization of zinc oxide with amine groups.**
- ✧ **OES and QMS investigation provide useful insights regarding the chemistry of the plasma reactor being useful in the optimization of the process.**
- ✧ **A balance between two competitive processes functionalization and etching has to be found to be able to optimize the plasma functionalization.**
- ✧ **XPS measurements correlated with chemical derivatization were employed to quantify the number of amine groups that can be bound on the surface of zinc oxide.**
- ✧ **The photoluminescence and dispersion properties of the zinc oxide can be enhanced in certain cases after plasma processing.**
- ✧ **Amine functional groups can be further used as reactive sites to connect with other biomolecules.**

Further investigations are still need to achieve the final step of the research when actual devices can be developed. There are still question about how the amine groups are actually bound to the surface of zinc oxide and how the functionalization process can be further improved. This thesis gives a univocal answer regarding these matters and some of these aspects will be considered in the foreseeable future.

References

[1] B. a Kairdolf, A. M. Smith, T. H. Stokes, M. D. Wang, A. N. Young, and S. Nie, "Semiconductor quantum dots for bioimaging and biodiagnostic applications.," *Annu. Rev. Anal. Chem. (Palo Alto. Calif).*, vol. 6, no. March, pp. 143–62, Jan. 2013.

[2] R. Freeman, X. Liu, and I. Willner, "Amplified multiplexed analysis of DNA by the exonuclease III-catalyzed regeneration of the target DNA in the presence of functionalized semiconductor quantum dots.," *Nano Lett.*, vol. 11, no. 10, pp. 4456–61, Oct. 2011.

[3] X. Michalet, F. F. Pinaud, L. a Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, a M. Wu, S. S. Gambhir, and S. Weiss, "Quantum dots for live cells, in vivo imaging, and diagnostics.," *Science*, vol. 307, no. 5709, pp. 538–44, Jan. 2005.

[4] A. Valizadeh, H. Mikaeili, M. Samiei, S. M. Farkhani, N. Zarghami, M. Kouhi, A. Akbarzadeh, and S. Davaran, "Quantum dots: synthesis, bioapplications, and toxicity.," *Nanoscale Res. Lett.*, vol. 7, no. 1, p. 480, Jan. 2012.

[5] C. Chen, A. Ogino, X. Wang, and M. Nagatsu, "Oxygen functionalization of multiwall carbon nanotubes by Ar/H2O plasma treatment☆," *Diam. Relat. Mater.*, vol. 20, no. 2, pp. 153–156, Feb. 2011.

[6] M. Král', A. Ogino, and M. Nagatsu, "Effect of hydrogen on amino group introduction onto the polyethylene surface by surface-wave plasma chemical modification," *J. Phys. D. Appl. Phys.*, vol. 41, no. 10, p. 105213, May 2008.

[7] Z. Ptb, Q. Ma, A. Ogino, T. Matsuda, and M. Nagatsu, "Synthesis of Nitrogen-doped ZnO Nanoparticles by Plasma- Enhanced Pulsed Laser Deposition," *Science (80-.).*, pp. 4–5.

[8] Q. Ou, T. Matsuda, M. Mesko, A. Ogino, and M. Nagatsu, "Cathodoluminescence Property of ZnO Nanophosphors Prepared by Laser Ablation," *Jpn. J. Appl. Phys.*, vol. 47, no. 1, pp. 389–393, Jan. 2008.

[9] A. K. C. Eds, *Further Titles of Interest Nanoparticles Molecular Clusters of the Main Group Elements Electrochemistry of Nanomaterials*. 2004, pp. 3–527.

[10] C. W. Bunn, "The lattice-dimensions of zinc oxide," *Proc. Phys. Soc.*, vol. 47, no. 5, pp. 835–842, Sep. 1935.

[11] Y. Review, "Raman Effect in," vol. 142, no. 2, 1966.

[12] G. Galli, “EPITAXIAL ZnO ON SAPPHIRE,” *Appl. Phys. Lett.*, vol. 16, no. 11, p. 439, 1970.

[13] Z. Chuan, “Handbook of Zinc Oxide and Related Materials.”

[14] A. Mang, “Reimann, and St. Riibenacke,” vol. 94, no. 4, pp. 251–254, 1995.

[15] Z. Fan and J. G. Lu, “Zinc oxide nanostructures: synthesis and properties.,” *J. Nanosci. Nanotechnol.*, vol. 5, no. 10, pp. 1561–73, Oct. 2005.

[16] D. C. Look, “Recent advances in ZnO materials and devices,” *Mater. Sci. Eng. B*, vol. 80, no. 1–3, pp. 383–387, Mar. 2001.

[17] U. Özgür, Y. I. Alivov, C. Liu, a. Teke, M. a. Reshchikov, S. Doğan, V. Avrutin, S.-J. Cho, and H. Morkoç, “A comprehensive review of ZnO materials and devices,” *J. Appl. Phys.*, vol. 98, no. 4, p. 041301, 2005.

[18] D. M. Bagnall, Y. F. Chen, Z. Zhu, T. Yao, S. Koyama, M. Y. Shen, T. Goto, and B. Zno, “Optically pumped lasing of ZnO at room temperature,” vol. 70, no. April, pp. 2230–2232, 1997.

[19] O. Pumped, U. Lasing, F. Zno, E. Science, Z. M. Gan, W. W. Lattice, F. Landolt-btrnstein, and N. York, “t Pergamon,” vol. 99, no. 12, pp. 873–875, 1996.

[20] H. Cao, Y. Zhao, S. Ho, E. Seelig, Q. Wang, and R. Chang, “Random Laser Action in Semiconductor Powder,” *Phys. Rev. Lett.*, vol. 82, no. 11, pp. 2278–2281, Mar. 1999.

[21] A. B. Greytak, C. J. Barrelet, Y. Li, and C. M. Lieber, “Semiconductor nanowire laser and nanowire waveguide electro-optic modulators,” *Appl. Phys. Lett.*, vol. 87, no. 15, p. 151103, 2005.

[22] A. Tsukazaki, A. Ohtomo, T. Onuma, M. Ohtani, T. Makino, M. Sumiya, K. Ohtani, S. F. Chichibu, S. Fuke, Y. Segawa, H. Ohno, H. Koinuma, and M. Kawasaki, “Repeated temperature modulation epitaxy for p-type doping and light-emitting diode based on ZnO,” *Nat. Mater.*, vol. 4, no. 1, pp. 42–46, Dec. 2004.

[23] Y. R. Ryu, W. J. Kim, and H. W. White, “Fabrication of homostructural ZnO p–n junctions,” *J. Cryst. Growth*, vol. 219, no. 4, pp. 419–422, Nov. 2000.

[24] L. J. Mandalapu, Z. Yang, F. X. Xiu, D. T. Zhao, and J. L. Liu, “Homojunction photodiodes based on Sb-doped p-type ZnO for ultraviolet detection,” *Appl. Phys. Lett.*, vol. 88, no. 9, p. 092103, 2006.

[25] O. Madelung, *Semiconductors - Basic Data*. Springer, 1996.

[26] A. Janotti and C. G. Van de Walle, "Fundamentals of zinc oxide as a semiconductor," *Reports Prog. Phys.*, vol. 72, no. 12, p. 126501, Dec. 2009.

[27] M. N. Kamalasanan and S. Chandra, "Sol-gel synthesis of ZnO thin films," *Thin Solid Films*, vol. 288, no. 1–2, pp. 112–115, Nov. 1996.

[28] W. L. Estrada, D. R. N. Acosta, E. Andrade, and M. Miki-yoshida, "Growth , structure and optical characterization of high quality ZnO thin ® lms obtained by spray pyrolysis," vol. 350, pp. 192–202, 1999.

[29] H. Funakubo, N. Mizutani, M. Yonetsu, A. Saiki, and K. Shinozaki, "Orientation Control of ZnO Thin Film Prepared by CVD," pp. 25–32, 2000.

[30] K. Sakurai, M. Kanehiro, K. Nakahara, T. Tanabe, S. Fujita, and S. Fujita, "E ! ects of oxygen plasma condition on MBE growth of ZnO," vol. 209, pp. 522–525, 2000.

[31] T. Yamamoto, T. Shiosaki, and A. Kawabata, "Characterization of ZnO piezoelectric films prepared by rf planar-magnetron sputtering," *J. Appl. Phys.*, vol. 51, no. 6, p. 3113, 1980.

[32] J. Molarius, J. Kaitila, T. Pensala, M. Ylilammi, V. T. T. Microelectronics, P. O. Box, and F.-V. T. T. Espoo, "Piezoelectric ZnO ® lms by r . f . sputtering," vol. 4, pp. 431–435, 1830.

[33] J. G. E. Gardeniers, Z. M. Rittersma, and G. J. Burger , "Preferred orientation and piezoelectricity in sputtered ZnO films," *J. Appl. Phys.*, vol. 83, no. 12, p. 7844, 1998.

[34] "1991_Smell sensor using zinc oxide thin films prepared by magnetron sputtering.pdf." .

[35] H. Nanto, H. Sokooshi, and T. Usuda, "Smell sensor using aluminium-doped zinc oxide thin film prepared by sputtering technique," *Sensors Actuators B Chem.*, vol. 10, no. 2, pp. 79–83, Jan. 1993.

[36] H. Nanto, T. Minami, and S. Takata, "Zinc-oxide thin-film ammonia gas sensors with high sensitivity and excellent selectivity," *J. Appl. Phys.*, vol. 60, no. 2, p. 482, 1986.

[37] A. K. Mukhopadhyay, P. Mitra, D. Chattopadhyay, H. S. Maiti, and C. Glass, "Influences of fabrication techniques and doping on hydrogen sensitivity of zinc oxide sensors," vol. 15, pp. 431–433, 1996.

[38] N. J. Dayan, S. . Sainkar, R. . Karekar, and R. . Aiyer, “Formulation and characterization of ZnO:Sb thick-film gas sensors,” *Thin Solid Films*, vol. 325, no. 1–2, pp. 254–258, Jul. 1998.

[39] J. Xu, Q. Pan, Y. Shun, and Z. Tian, “Grain size control and gas sensing properties of ZnO gas sensor,” *Sensors Actuators B Chem.*, vol. 66, no. 1–3, pp. 277–279, Jul. 2000.

[40] J. F. Chang, H. H. Kuo, I. C. Leu, and M. H. Hon, “The effects of thickness and operation temperature on ZnO:Al thin film CO gas sensor,” *Sensors Actuators B Chem.*, vol. 84, no. 2–3, pp. 258–264, May 2002.

[41] J. Xu, “Luminescence in ZnO,” no. December, 2004.

[42] P. A. Rodnyi and I. V Khodyuk, “Optical and Luminescence Properties of Zinc Oxide,” vol. 111, no. 5, pp. 776–785, 2011.

[43] K. Vanheusden, C. H. Seager, W. L. Warren, D. R. Tallant, and J. a. Voigt, “Correlation between photoluminescence and oxygen vacancies in ZnO phosphors,” *Appl. Phys. Lett.*, vol. 68, no. 3, p. 403, 1996.

[44] a. B. DjurišicÏ, Y. H. Leung, K. H. Tam, L. Ding, W. K. Ge, H. Y. Chen, and S. Gwo, “Green, yellow, and orange defect emission from ZnO nanostructures: Influence of excitation wavelength,” *Appl. Phys. Lett.*, vol. 88, no. 10, p. 103107, 2006.

[45] A. B. Djurisić and Y. H. Leung, “Optical properties of ZnO nanostructures.,” *Small*, vol. 2, no. 8–9, pp. 944–61, Aug. 2006.

[46] L. K. Adams, D. Y. Lyon, and P. J. J. Alvarez, “Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions.,” *Water Res.*, vol. 40, no. 19, pp. 3527–32, Nov. 2006.

[47] Z. Huang, X. Zheng, D. Yan, G. Yin, X. Liao, Y. Kang, Y. Yao, D. Huang, and B. Hao, “Toxicological effect of ZnO nanoparticles based on bacteria.,” *Langmuir*, vol. 24, no. 8, pp. 4140–4, Apr. 2008.

[48] J. Zhou, N. S. Xu, and Z. L. Wang, “Dissolving Behavior and Stability of ZnO Wires in Biofluids: A Study on Biodegradability and Biocompatibility of ZnO Nanostructures,” *Adv. Mater.*, vol. 18, no. 18, pp. 2432–2435, Sep. 2006.

[49] N. Jones, B. Ray, K. T. Ranjit, and A. C. Manna, “Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms.,” *FEMS Microbiol. Lett.*, vol. 279, no. 1, pp. 71–6, Mar. 2008.

[50] E. Comini, G. Faglia, G. Sberveglieri, Z. Pan, and Z. L. Wang, “Stable and highly sensitive gas sensors based on semiconducting oxide nanobelts,” *Appl. Phys. Lett.*, vol. 81, no. 10, p. 1869, 2002.

[51] X. D. Bai, P. X. Gao, Z. L. Wang, and E. G. Wang, “Dual-mode mechanical resonance of individual ZnO nanobelts,” *Appl. Phys. Lett.*, vol. 82, no. 26, p. 4806, 2003.

[52] W. L. Hughes and Z. L. Wang, “Nanobelts as nanocantilevers,” *Appl. Phys. Lett.*, vol. 82, no. 17, p. 2886, 2003.

[53] X. Y. Kong and Z. L. Wang, “Spontaneous Polarization-Induced Nanohelixes, Nanosprings, and Nanorings of Piezoelectric Nanobelts,” *Nano Lett.*, vol. 3, no. 12, pp. 1625–1631, Dec. 2003.

[54] X. Y. Kong, Y. Ding, R. Yang, and Z. L. Wang, “Single-crystal nanorings formed by epitaxial self-coiling of polar nanobelts.,” *Science*, vol. 303, no. 5662, pp. 1348–51, Mar. 2004.

[55] S. Evolution, *Functional and Smart Materials*. 1998.

[56] Z. L. Wang, “Nanostructures of zinc oxide,” no. June, pp. 26–33, 2004.

[57] T. Sekiguchi, S. Miyashita, K. Obara, T. Shishido, and N. Sakagami, “Hydrothermal growth of ZnO single crystals and their optical characterization,” *J. Cryst. Growth*, vol. 214–215, pp. 72–76, Jun. 2000.

[58] N. Sakagami, M. Yamashita, T. Sekiguchi, S. Miyashita, K. Obara, and T. Shishido, “Variation of electrical properties on growth sectors of ZnO single crystals,” *J. Cryst. Growth*, vol. 229, no. 1–4, pp. 98–103, Jul. 2001.

[59] W. Li, E. Shi, W. Zhong, and Z. Yin, “Growth mechanism and growth habit of oxide crystals,” vol. 203, no. July 1998, pp. 186–196, 1999.

[60] E. Ohshima, H. Ogino, I. Niikura, K. Maeda, M. Sato, M. Ito, and T. Fukuda, “Growth of the 2-in-size bulk ZnO single crystals by the hydrothermal method,” *J. Cryst. Growth*, vol. 260, no. 1–2, pp. 166–170, Jan. 2004.

[61] M. Shiloh and J. Gutman, “Growth of ZnO single crystals by chemical vapour transport*1,” *J. Cryst. Growth*, vol. 11, no. 2, pp. 105–109, 1971.

[62] J. R. Look, D.C., Reynolds, D.C., Sizelove, G. and Jones, R.L., Litton, C.W., Cantwell, and W. C. Harsch, “ELECTRICAL PROPERTIES OF BULK ZnO,” vol. 1098, no. 97, pp. 399–401, 1998.

[63] J. E. Nause, “ZnO broadens the wectrum,” vol. 12, no. 4, pp. 28–31, 1999.

[64] J. Nause and B. Nemeth, “Pressurized melt growth of ZnO boules,” *Semicond. Sci. Technol.*, vol. 20, no. 4, pp. S45–S48, Apr. 2005.

[65] Z. R. Tian, J. a Voigt, J. Liu, B. McKenzie, M. J. McDermott, M. a Rodriguez, H. Konishi, and H. Xu, “Complex and oriented ZnO nanostructures.,” *Nat. Mater.*, vol. 2, no. 12, pp. 821–6, Dec. 2003.

[66] N. Dasgupta, R. Paily, A. Dasgupta, and P. Misra, “PULSED LASER DEPOSITION - A REVIEW Pulsed Laser Deposition for MOS Gate Dielectric Films,” *Appl. Surf. Sci.*, pp. 7–78, 2005.

[67] a. V. Singh, R. M. Mehra, N. Buthrath, A. Wakahara, and A. Yoshida, “Highly conductive and transparent aluminum-doped zinc oxide thin films prepared by pulsed laser deposition in oxygen ambient,” *J. Appl. Phys.*, vol. 90, no. 11, p. 5661, 2001.

[68] L. P. L. Ablation, L. Phase, and P. Laser, “Liquid-Phase Pulsed Laser Ablation,” pp. 33–51, 1987.

[69] S. P. and P. G. S. N. J. Ianno, L. McConville, N. Shaikh, “Characterization of pulsed laser deposited zinc oxide,” vol. 220, pp. 92–99, 1992.

[70] P. Couvreur, R. Gref, K. Andrieux, and C. Malvy, “Nanotechnologies for drug delivery: Application to cancer and autoimmune diseases,” *Prog. Solid State Chem.*, vol. 34, no. 2–4, pp. 231–235, Jul. 2006.

[71] I. Brigger, C. Dubernet, and P. Couvreur, “Nanoparticles in cancer therapy and diagnosis.,” *Adv. Drug Deliv. Rev.*, vol. 54, no. 5, pp. 631–51, Sep. 2002.

[72] Y. Pathak and D. Thassu, *Drug Delivery Nanoparticles Formulation and Characterization*, vol. 191. .

[73] P. J. Cassidy and G. K. Radda, “Molecular imaging perspectives.,” *J. R. Soc. Interface*, vol. 2, no. 3, pp. 133–44, Jun. 2005.

[74] W. C. Zhang, X. L. Wu, H. T. Chen, J. Zhu, and G. S. Huang, “Excitation wavelength dependence of the visible photoluminescence from amorphous ZnO granular films,” *J. Appl. Phys.*, vol. 103, no. 9, p. 093718, 2008.

[75] A. M. Smith, G. Ruan, M. N. Rhyner, and S. Nie, “Engineering luminescent quantum dots for in vivo molecular and cellular imaging.,” *Ann. Biomed. Eng.*, vol. 34, no. 1, pp. 3–14, Jan. 2006.

[76] M. E. Akerman, W. C. W. Chan, P. Laakkonen, S. N. Bhatia, and E. Ruoslahti, “Nanocrystal targeting in vivo.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 99, no. 20, pp. 12617–21, Oct. 2002.

[77] M. Bruchez, M. Moronne, P. Gin, S. Weiss, and a P. Alivisatos, “Semiconductor nanocrystals as fluorescent biological labels.,” *Science*, vol. 281, no. 5385, pp. 2013–6, Sep. 1998.

[78] W. C. Chan, “Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection,” *Science (80-.).*, vol. 281, no. 5385, pp. 2016–2018, Sep. 1998.

[79] S. Kim, Y. T. Lim, E. G. Soltesz, A. M. De Grand, J. Lee, A. Nakayama, J. A. Parker, T. Mihaljevic, R. G. Laurence, D. M. Dor, L. H. Cohn, M. G. Bawendi, and J. V Frangioni, “Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping.,” *Nat. Biotechnol.*, vol. 22, no. 1, pp. 93–7, Jan. 2004.

[80] M. Hassan and B. A. Klaunberg, “Overview Biomedical Applications of Fluorescence Imaging In Vivo,” vol. 54, no. 6, pp. 635–644, 2004.

[81] D. K. Yi, S. T. Selvan, S. S. Lee, G. C. Papaefthymiou, D. Kundaliya, and J. Y. Ying, “Silica-coated nanocomposites of magnetic nanoparticles and quantum dots.,” *J. Am. Chem. Soc.*, vol. 127, no. 14, pp. 4990–1, Apr. 2005.

[82] X. Zhong, Y. Feng, W. Knoll, and M. Han, “Alloyed Zn(x)Cd(1-x)S nanocrystals with highly narrow luminescence spectral width.,” *J. Am. Chem. Soc.*, vol. 125, no. 44, pp. 13559–63, Nov. 2003.

[83] Z. Li, Y. Zhang, and S. Jiang, “Multicolor Core/Shell-Structured Upconversion Fluorescent Nanoparticles,” *Adv. Mater.*, vol. 20, no. 24, pp. 4765–4769, Dec. 2008.

[84] L. Tušek, M. Nitschke, C. Werner, K. Stana-Kleinschek, and V. Ribitsch, “Plasma-activated immobilization of biomolecules onto graphite-encapsulated magnetic nanoparticles,” *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 195, no. 1–3, pp. 81–95, Dec. 2001.

[85] M. Colombo, S. Carregal-Romero, M. F. Casula, L. Gutiérrez, M. P. Morales, I. B. Böhm, J. T. Heverhagen, D. Prosperi, and W. J. Parak, “Biological applications of magnetic nanoparticles.,” *Chem. Soc. Rev.*, vol. 41, no. 11, pp. 4306–34, Jun. 2012.

[86] J. Zhou, Z. Liu, and F. Li, “Upconversion nanophosphors for small-animal imaging.,” *Chem. Soc. Rev.*, vol. 41, no. 3, pp. 1323–49, Feb. 2012.

[87] K. S. Siow, L. Britcher, S. Kumar, and H. J. Griesser, “Plasma Methods for the Generation of Chemically Reactive Surfaces for Biomolecule Immobilization and Cell Colonization - A Review,” *Plasma Process. Polym.*, vol. 3, no. 6–7, pp. 392–418, Aug. 2006.

[88] L. Detomaso, R. Gristina, R. d'Agostino, G. S. Senesi, and P. Favia, "Plasma deposited acrylic acid coatings: Surface characterization and attachment of 3T3 murine fibroblast cell lines," *Surf. Coatings Technol.*, vol. 200, no. 1–4, pp. 1022–1025, Oct. 2005.

[89] T.-J. Hsueh, S.-J. Chang, C.-L. Hsu, Y.-R. Lin, and I.-C. Chen, "Highly sensitive ZnO nanowire ethanol sensor with Pd adsorption," *Appl. Phys. Lett.*, vol. 91, no. 5, p. 053111, 2007.

[90] S. Elhag, Z. H. Ibupoto, O. Nur, and M. Willander, "Incorporating β-cyclodextrin with ZnO nanorods: a potentiometric strategy for selectivity and detection of dopamine.," *Sensors (Basel).*, vol. 14, no. 1, pp. 1654–64, Jan. 2013.

[91] J. Lee, B. S. Kang, B. Hicks, T. F. Chancellor, B. H. Chu, H.-T. Wang, B. G. Keselowsky, F. Ren, and T. P. Lele, "The control of cell adhesion and viability by zinc oxide nanorods.," *Biomaterials*, vol. 29, no. 27, pp. 3743–9, Sep. 2008.

[92] S. M. Al-Hilli, R. T. Al-Mofarji, and M. Willander, "Zinc oxide nanorod for intracellular pH sensing," *Appl. Phys. Lett.*, vol. 89, no. 17, p. 173119, 2006.

[93] a. Wei, X. W. Sun, J. X. Wang, Y. Lei, X. P. Cai, C. M. Li, Z. L. Dong, and W. Huang, "Enzymatic glucose biosensor based on ZnO nanorod array grown by hydrothermal decomposition," *Appl. Phys. Lett.*, vol. 89, no. 12, p. 123902, 2006.

[94] O. Taratula, E. Galoppini, R. Mendelsohn, P. I. Reyes, Z. Zhang, Z. Duan, J. Zhong, and Y. Lu, "Stepwise Functionalization of ZnO Nanotips with DNA," no. 13, pp. 2107–2113, 2009.

[95] Z. Zhang, H. Chen, J. Zhong, G. Saraf, and Y. Lu, "Fast and Reversible Wettability Transitions on ZnO Nanostructures," *J. Electron. Mater.*, vol. 36, no. 8, pp. 895–899, May 2007.

[96] S. M. Al-Hilli, M. Willander, A. Öst, and P. Strålfors, "ZnO nanorods as an intracellular sensor for pH measurements," *J. Appl. Phys.*, vol. 102, no. 8, p. 084304, 2007.

[97] R. Yoshida, D. Kitamura, and S. Maenosono, "Mutagenicity of water-soluble ZnO nanoparticles in Ames test.," *J. Toxicol. Sci.*, vol. 34, no. 1, pp. 119–22, Feb. 2009.

[98] M. H. Asif, a. Fulati, O. Nur, M. Willander, C. Brännmark, P. Strålfors, S. I. Börjesson, and F. Elinder, "Functionalized zinc oxide nanorod with ionophore-membrane coating as an intracellular Ca[sup 2+] selective sensor," *Appl. Phys. Lett.*, vol. 95, no. 2, p. 023703, 2009.

[99] S. Al-Hilli and M. Willander, “Membrane potential measurements across a human fat cell using ZnO nanorods.,” *Nanotechnology*, vol. 20, no. 17, p. 175103, Apr. 2009.

[100] A. Dorfman, N. Kumar, and J. Hahm, “Highly sensitive biomolecular fluorescence detection using nanoscale ZnO platforms.,” *Langmuir*, vol. 22, no. 11, pp. 4890–5, May 2006.

[101] R. K. Dutta, P. K. Sharma, and A. C. Pandey, “Surface Enhanced Raman Spectra Of Escherichia Coli Cells,” vol. 4, no. 1, pp. 83–87, 2009.

[102] E. Galoppini, “Linkers for anchoring sensitizers to semiconductor nanoparticles,” *Coord. Chem. Rev.*, vol. 248, no. 13–14, pp. 1283–1297, Jul. 2004.

[103] O. Taratula, E. Galoppini, D. Wang, D. Chu, Z. Zhang, H. Chen, G. Saraf, and Y. Lu, “Binding studies of molecular linkers to ZnO and MgZnO nanotip films.,” *J. Phys. Chem. B*, vol. 110, no. 13, pp. 6506–15, Apr. 2006.

[104] A. Einstein, “Concerning an Heuristic Point of View Toward the Emission and Transformation of Light 1 . Concerning a Difficulty with Regard to the Theory of Blackbody Radiation,” pp. 1–16, 1965.

[105] C. F. Meares, “Bioconjugate Chemistry,” pp. 93–98, 2003.

[106] M. A. Ciolan, I. Motrescu, D. Luca, and M. Nagatsu, “Low Temperature Plasma Functionalization Of ZnO With Amine Groups For Bioapplications,” *DNJB*, vol. 9, no. 2, pp. 483–491, 2014.

[107] H. Search, C. Journals, A. Contact, M. Iopscience, and I. P. Address, “Surface Wave Eigenmodes in a Finite-Area Plane Microwave Plasma,” vol. 337.

[108] S. Wave, “A Waveguiide-Based Launcher to Sustain Long Plasma Columns Through the Propagation of an Electromagnetic Surface Wave,” vol. 48, no. 3, 1984.

[109] H. Search, C. Journals, A. Contact, M. Iopscience, P. S. Sci, and I. P. Address, “High-density flat plasma production based on surface waves,” vol. 192, 1998.

[110] M. A. Ciolan, I. Motrescu, D. Luca, and M. Nagatsu, “Mass spectrometric study of Ar / NH 3 surface wave plasma utilized for surface functionalization of ZnO nanoparticles,” *Jpn. J. Appl. Phys.*, vol. 010207, no. 53, pp. 1–5, 2014.

[111] L. Tušek, M. Nitschke, C. Werner, K. Stana-Kleinschek, and V. Ribitsch, “Amino group introduction onto multiwall carbon nanotubes by NH3/Ar plasma

treatment," *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 195, no. 1–3, pp. 81–95, Dec. 2001.

[112] L. Tušek, M. Nitschke, C. Werner, K. Stana-Kleinschek, and V. Ribitsch, "Optimization of Amino Group Introduction onto Polyurethane Surface Using Ammonia and Argon Surface-Wave Plasma," *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 195, no. 1–3, pp. 81–95, Dec. 2001.

[113] K. L. Busch, "Mass Spectrometry," pp. 145–158, 2003.

Activity

Journal articles

1. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, and Masaaki Nagatsu (2014) Low Temperature Plasma Functionalization of ZnO with Amine Groups for Bioapplications. In "Digest Journal of Nanomaterials and Biostructures", page: 483 – 491, 2014.
2. Mihai Alexandru Ciolan, Iuliana Motrescu, Dumitru Luca, and Masaaki Nagatsu (2014) Mass spectrometric study of Ar/NH_3 surface wave plasma utilized for surface functionalization of ZnO nanoparticles. In "Japanese Journal of Applied Physics", 010207-1 - 010207-5.

Conferences

1. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Akihisa Ogino, Dumitru Luca, Masaaki Nagatsu "Functional Amino Group Addition to Zinc Oxide Nanoparticles by Plasma Processing", poster The 5Th International Conference on Plasma-Nanotechnology and Science (IC-PLANTS) 2012, Freude, Inuyama, Japan, March 9-10.
2. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Akihisa Ogino, Dumitru Luca, Masaaki Nagatsu – "Plasma Surface Modification of ZnO Nanoparticles for Bio-Imaging Applications", The 58th Spring Meeting of the Japan Society of Applied Physics, Waseda University, Tokyo, 15-18 March 2012, oral presentation.
3. Iuliana Motrescu, **Mihai Alexandru Ciolan**, Akihisa Ogino, and Masaaki Nagatsu – "Bio-molecules plasma processing for bioimaging applications", The 58th Spring Meeting of the Japan Society of Applied Physics, Waseda University, Tokyo, 15-18 March 2012, oral presentation.

4. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Akihisa Ogino, Dumitru Luca, Masaaki Nagatsu, ”Plasma Surface Modification of ZnO Nanoparticles for Bio-Imaging Applications” The 58th Spring Meeting of the Japan Society of Applied Physics, Waseda University, Tokyo, 15-18 March 2012, oral presentation 17p-B8-12.
5. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, ”Low Temperature Plasma Processing of ZnO Nanostructures Produced by Pulsed Laser Ablation for Bio-Applications”, Proceedings of the 11th Conference on Global Research and Education in Engineers for Better Life, 27-30 August, Budapest, Hungary, 321-329.
6. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, ”Amine Groups Functionalization of ZnO Produced by Pulsed Laser Ablation”, The 59th JSAP Autumn Meeting, 11-14 September 2012, Ehime University and Matsuyama University, Matsuyama, Japan.
7. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, ”Functionalization of Zinc Oxide Nanoparticles Obtained by Pulsed Laser Ablation Technique”, IUMRS – ICEM 2012 – International Conference on Electronic Materials, 23-28 September 2012, Yokohama, Japan.
8. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, ”Ammonia excited surface wave plasma processing of zinc oxide nanomaterials for amino group functionalisation” The 11th Asia-Pacific Conference on Plasma Science and Technology and 25th Symposium on Plasma Science for Materials, 2-5 October 2012, Kyoto, Japan.
9. Iuliana Motrescu, **Mihai Alexandru Ciolan**, Dumitru Luca, Masaaki Nagatsu, ”Plasma Surface Functionalization of ZnO Nanoparticles for Bio-Imaging Applications”, The 14th Takayanagi Kenjiro Memorial Symposium, 27-28 November 2012, Shizuoka University, Hamamatsu, Japan.
10. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, “Luminescence and dispersion properties of plasma-modified ZnO

nanoparticles for bio-imaging”, 29th Annual Meeting of The Japan Society of Plasma Science and Nuclear Fusion Research (JSPF 2012), 27-30 November 2012, Clover Plaza Kasuga, Fukuoka, Japan.

11. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, ”The Role of Plasma Processing of ZnO for Amino Groups Functionalization”, The 5th International Symposium on Advanced Plasma Science and its Applications for Nitrides and Nanomaterials, ISPlasma 2013, January 28 – February 1, 2013, Nagoya University, Japan.
12. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, “Effect of Ammonia Plasma Treatment on Photoluminescent and Crystalline Property of ZnO”, 30th Symposium on Plasma Processing, Hamamatsu, Japan, January 21st-23rd, 2013 (oral presentation) p.85.
13. Iuliana Motrescu, **Mihai Alexandru Ciolan**, Dumitru Luca, Masaaki Nagatsu, “Nitrogen Plasma Modification of Amino Acids with or without ZnO Nanoparticles”, The 60th JSAP Spring Meeting, Kanagawa Institute of Technology, Japan, March 27th-30th, 2013 (poster presentation).
14. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, “Characterization of plasma functionalized ZnO nanoparticles”, The 60th JSAP Spring Meeting, Kanagawa Institute of Technology, Japan, March 27th-30th, 2013 (poster presentation).
15. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, “Surface Amination of ZnO Nanoparticles by Surface-Wave Excited Ar/NH3 Plasma Processing”, 16th International Conference on Plasma Physics and Applications, Magurele, Romania, June 20th-25th, 2013 (poster presentation).
16. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, “Surface functionalization of ZnO nanoparticles with Ar/NH3 surface-wave plasma for bioimaging applications”, The 35th International Symposium on Dry Process (DPS 2013),Jeju Island South Korea, August 29-30, 2013, P42.

17. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Biofunctionalization of ZnO Nanoparticles Modified by Ammonia Plasma for Bioimaging Applications", The 2013 JSAP-MRS, 16-20 September 2013, Doshisha University, Kyoto, Japan, 17p-PM4-23.
18. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Influence of Ar/NH3 excited surface wave plasma processing on the photoluminescence properties of ZnO nanoparticles", 12th International Conference on Global Research and Education, inter-Academia 2013, Sofia, Bulgaria, 23-27 September 2013.*
19. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Study of the Optical Properties of ZnO Nanoparticles Functionalized by Plasma Processing", The 15th Takayanagi Kenjiro Memorial Symposium, 12-13 November 2013, Hamamatsu, Shizuoka University.
20. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Spectrophotometric study for quantifying the amino groups bonded to the zinc oxide nanoparticles by plasma processing", 23rd Annual Meeting of MRS-J, Advanced Materials Researches Breakthrough to the Innovations for Ecology and Energy, International Symposium Frontier of Nano-Materials Based on Advanced Plasma Technologies, 9-11 December 2013, Yokohama, Japan, P-09-010.
21. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Optical detection of sugar chains connected to plasma aminated zinc oxide nanomaterials", 30th Annual Meeting of The Japan Society of Plasma Science and Nuclear Fusion Research (JSPF 2013), 3-6 December 2013, Tokyo Institute of Technology.
22. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, „Quantification of amine groups introduced on the surface of zinc oxide nanoparticles by Ar/NH3 plasma processing", 8th International Conference on

Reactive Plasmas 31st Symposium on Plasma Processing, February 3-7, 2014 Fukuoka Convention Center, Fukuoka, Japan, 6P-PM-S14-P31.

23. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, „Molecule immobilization on amine enriched zinc oxide by plasma processing", 6th International Symposium on Advanced Plasma Science and its Applications for Nitrides and Nanomaterials (ISPlasma 2014), 7th International Conference on Plasma-Nano Technology and Science(IC-PLANTS 2014), March 2-6, 2014, Meijo University, Nagoya, Japan.
24. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Evaluation of amine functionalities bound on the ZnO surface by plasma processing used for biomolecule detection", The 61st Spring Meeting of the Japan Society of Applied Physics, Aoyama Gakuin University, Tokyo, 17-20 March 2014, 19A-PA4-7.
25. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Novel approach for zinc oxide nanomaterials functionalization based on dry plasma processing", The 41st IEEE International Conference on Plasma Science and the 20th International Conference on High-Power Particle Beams (ICOPS/BEAMS2014), May 25 – 29, 2014, Marriott Wardman Park, Washington DC.
26. **Mihai Alexandru Ciolan**, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, "Bioimmobilization of ZnO Nanoparticles Functionalized by Surface-wave Plasma Processing", 10th International Conference On Physics Of Advanced Materials (ICPAM-10), 22-28 September 2014, Iasi, Romania.

Awards

1. Young Scientist Award for Plasma Application 2012 – The Japan Society of Plasma Science and Nuclear Fusion Research
2. First prize Young Scientist Award interAcademia 2013 – Sofia, Bulgaria

Acknowledgements

I would like to express my gratitude to all the people who helped me achieve my goals for my PhD thesis.

First and foremost I am grateful to my supervisor, Professor Massaki Nagatsu. Through his full support during my study period here in Japan, his expertise, kind understanding, patience, and vast knowledge, I was able to evolve as a researcher. His guidance and him believing in me always got me on the right track.

Same gratitude goes to my Romanian supervisor, Professor Dumitru Luca, that made this doctoral stage possible, giving me the chance to study in this marvelous place - Japan. He has been an inspiration for myself and I truly appreciate his guidance, advices, and encouragements for pursuing this road.

Another very special person to me is Dr. Iuliana Motrescu to whom I like to bring my sincere and grateful acknowledgments. She thought me how to deal with the challenges that live had to offer, not only regarding the scientific world but also for daily living. She unconditionally guided my work and encouraged me perform the best I can in my research. I shall always appreciate and keep in my mind and hearth all the beautiful moments.

I wish to extend my gratitude to Mr. Waichi Tomoda, IJRC, Shizuoka University for his guidance and help during operation of analyzing devices and Professor Hiroko Kominami and Assistant Professor Atsushi Nakamura from Research Institute of Electronics for photoluminescence measurements assistance.

All of these would not be possible without the financial support of my study offered by the Ministry of Education, Culture, Sports, Science and Technology of Japanese Government through the MEXT scholarship.

I must thank all the laboratory members, whom I consider friends now for their help and the pleasant environment they created in the lab, especially Dr. Raman Bekarevich with whom I always had the chance to find solution to diverse technical challenges. I truly appreciate the nice moments spent with him and my good friend Rangga Winantyo too. I feel lucky to have met them both and have them as friends.

Even if Japan is located far away from Romania I always felt the unconditional love and support of my family that gave me strength to achieve my goals and fulfill my dreams, so I need to thank them for that.

For the past three years I had the chance to meet many people here in Japan and it is impossible for me to thank them all. I would like to apologize for those unmentioned, and assure them that they will always be remembered.

Last but not least I would like to show my appreciation towards the referees of this thesis: Professor Tetsu Mieno, Professor Hisao Suzuki and Professor Kazuhiko Hara for their precious time and energy spent for understanding its content and providing me valuable feedback.