UNIVERSITATEA „ALEXANDRU IOAN CUZA“ din IAŞI

FACULTATEA de FIZICĂ

PhD thesis sumary

Surface modification of zinc oxide nanoparticles and films by low-pressure plasma processing

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- Zinc oxide materials
- Plasma functionalization
- Amine groups
- Bioimaging

Contents

Introduction

Bioimaging offers 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. Materials biocompatibility is a necessary property of materials during their interaction with biological systems.

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 its optical properties, as photoluminescence, at room temperature. There is a lack of reports regarding the use of zinc oxide based materials for imaging. Till now, reports about the functionalization of zinc oxide by wet chemical methods were published but these methods have major drawbacks when dealing with materials that should be compatible with living organism. They feature increased toxicity due to the chemicals used for functionalization, agglomeration of the nanoparticles, low dispersion in aqueous media and so on.

This thesis consists of 6 main chapters and a section of Conclusions. Chapter 1 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 2 provides brief information regarding the functionalization of nanomaterials and current advances in bioimaging applications. Chapter 3 introduces the methods employed during this study to analyze and characterize the outcome of the plasma processing in term of functionalization. Chapters 4,5 and 6 are dedicated to original results. Chapter 4 includes original results regarding the functionalization of the zinc oxide materials and also about the surface wave plasma diagnosis by different measurements. These results play a key role for the understanding how plasma functionalization works and how the process can be optimized. The possibility of adding functional groups on the surface of zinc oxide, correlated with the plasma parameters, is presented in detail in Chapter 5. Optimization of the processing techniques was 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 analyzed further more. Chapter 6 deals with the production of zinc oxide thin films by reactive magnetron sputtering, as well as the investigation of the optical and crystallographic properties of the plasma functionalized thin films. X-ray diffraction methods, along with scanning electron microscopy (SEM), photoluminescence and fluorescence techniques are used to investigate the materials. Chapter 7 summarizes the key points of the thesis.

Chapter 1. Nanomaterials

Zinc oxide is a II-VI semiconductor 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. Until now the growth of zinc oxide was done by employing diverse techniques like: chemical vapor deposition (CVD), DC and RF sputtering , molecular beam epitaxy (MBE), pulsed laser deposition (PLD). Due to its low toxicity compared with other semiconductor based materials, we promote the use of zinc oxide for the development of novel bioimaging techniques.

Chapter 2. Functionalization of nanomaterials

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.

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 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. 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.

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. 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.

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. 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. Conventional wet-chemistry approaches employed for changing the physical–chemical characteristics of material surfaces often have severe 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 means, and accordingly, the plasma approach was considered for the generation of biocompatible surfaces. Plasma-surface modification is an effective and economical treatment technique for many materials and of growing interest 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.

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. Gas molecules activated by plasma and the created species inside plasma interact with the surface of the materials and special functions such as hydroxyl, carboxyl, carbonyl and amino groups can be bonded at the surface of the nanoparticles.

The process of functionalization of zinc oxide based nanostructures by wet chemical methods was described in many reports over the years. 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.

Chapter 3. Experimental methods

In this chapter experimental methods used for the production and characterization of nanomaterials are described. The methods used are: X-ray Photoelectron Spectroscopy (XPS), Transmission Electron microscopy (TEM), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), chemical derivatization methods, fluorescence microscopy, photoluminescence measurements.

The production of zinc oxide nanostructures is realized by pulsed laser deposition with a NdYAG laser (1064nm) and radio frequency magnetron sputtering.

The functionalization of nanoparticles and thin films is achieved by employing low pressure surface wave excited plasma (SWP).

Chapter 4. Plasma functionalization of zinc oxide nanostructures

We started our study with the idea in mind to promote zinc oxide as suitable material for the development of new bioimaging application.

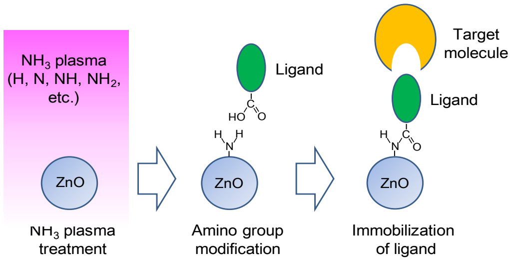

Figure 1.Illustration of biofunctionalization of zinc oxide nanoparticles and utilization in bioapplications.

As it can be seen from Figure 1, 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.

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. Plasma processing was done in pure ammonia excited SWP.

OES measurement were preformed to prove the existence of a proper reactive medium for amine functionalization as seen in Figure 2. 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.

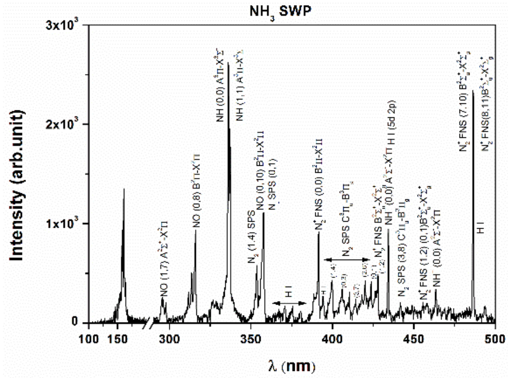

Figure 2.OES spectra of pure ammonia excites SWP.

The result of functionalization was investigated by monitoring the N 1s XPS spectra of the untreated zinc oxide micro-particles and the zinc oxide samples treated by ammonia as seen in Figure 3. 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.

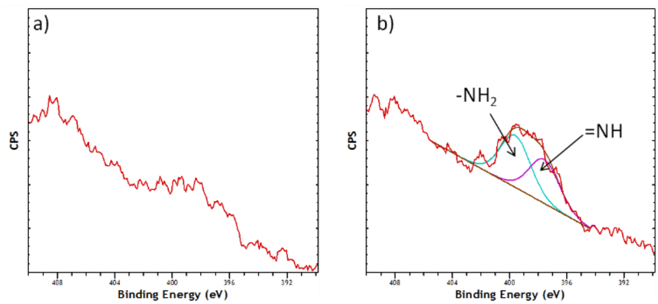

Figure 3.XPS spectra of N 1s for a) untreated ZnO particles and b) ZnO particles treated by ammonia SWP.

For a further confirmation and quantification of the amine and amide functionalities a chemical method: ninhydrin assay was used. The quantification of the method is done using a spectrophotometer.

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.

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. Nanoparticles with controlable size and good crystalinity are obtained as seen from Figure 4.

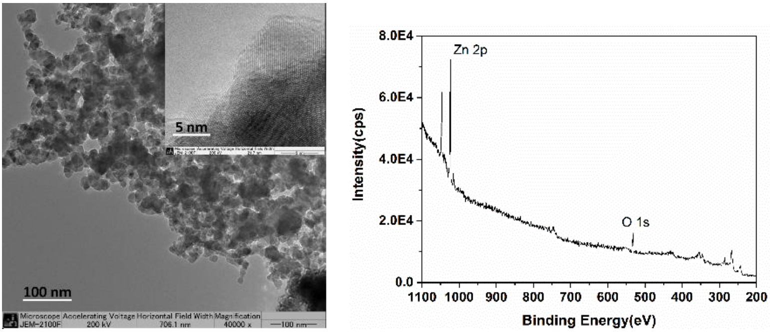

Figure 4.TEM image and XPS spectra of zinc oxide nanoparticles fabricated by pulsed laser ablation

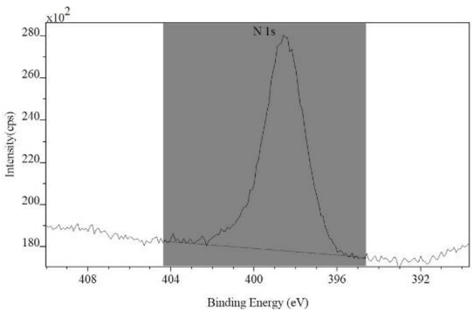

Figure 5.40 XPS spectrum of N 1s for ZnO nanoparticles.

I employed once again the XPS analysis and, as it can be seen from Figure 5, the nitrogen content is increasing for the processed sample in surface wave plasma ignited for grid power of 700 W.

A complementary method to check functionalities by connecting them to a fluorescent dye and then imaging the sample at the fluorescent microscope (Figure 6). As a fluorescent dye I used Alexa Fluor 488 that specifically connects amine groups.

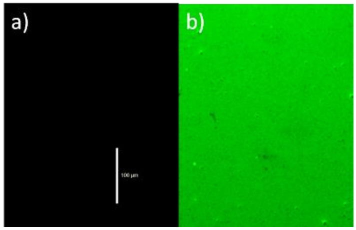

Figure 6.Fluorescent microscope images: a) untreated, and b) SWP processed zinc oxide nanomaterials.

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

Chapter 5. Optimization of the plasma processing for amine group functionalization of ZnO materials

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.

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 7 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.

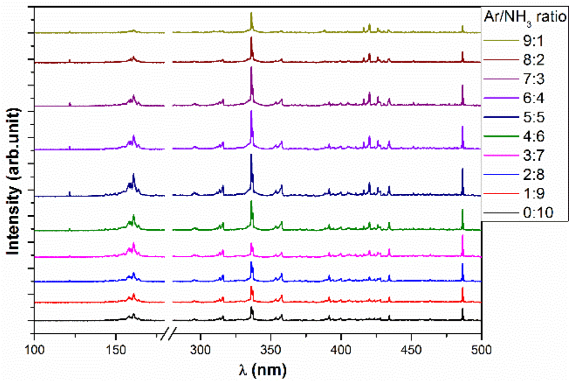

Figure 7. OES spectra comparison for different mixtures of Ar and NH_3 used to excite SWP.

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 8. 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 understand the machanism of plasma fucntionalization of zinc oxide nanostructures we used a Hidden Analitycal PMS003 high resolution quadrupole mass spectrometer. Figure 9 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.

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.

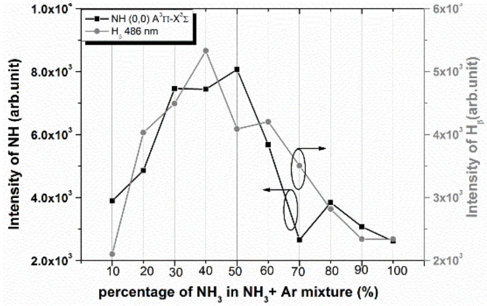

Figure 8. Comparative plotting of the emission intensity of the NH and Hβ lines for different mixtures of NH_3/Ar used to generate the SWP.

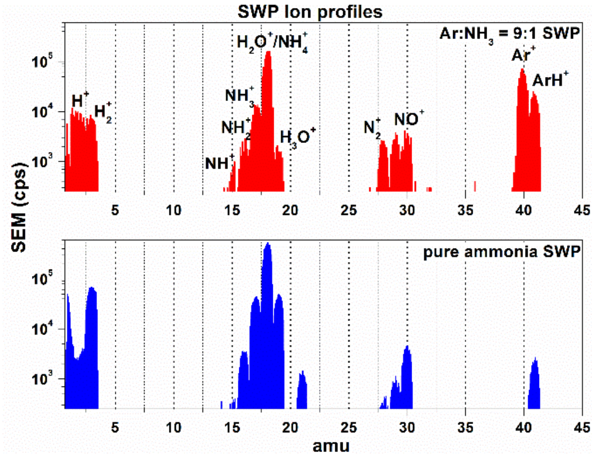

Figure 9. Comparative plotting of ion profiles in pure NH_3 SWP and mixture of 10% NH_3 and 90% Ar SWP.

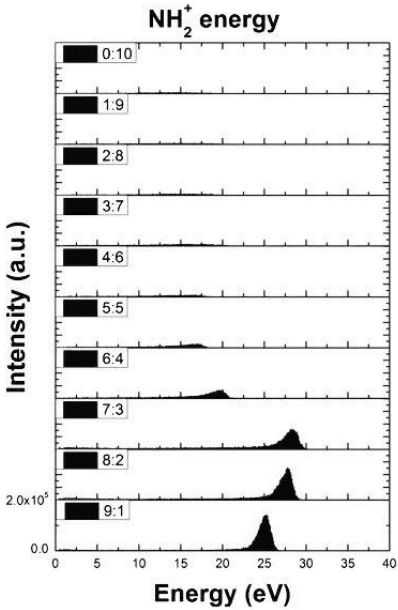

Figure 10. Ionic energy profiles of different mixtures of NH_3/Ar.]

To quantify the reactive sites, represented by the amine groups, a chemical derivatization method is employed. 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. The SPDP crosslinkers connect with the amine groups and the new created bonds are the cleaved with the help of a reducing agent. 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. Pyridine is a chromofore and thus, its absorbance at 343nm can be used to calculate the number of amine groups in the samples. 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 (Figure 11), the maximum is obtained for the 30% NH3 and 70% Ar. Once again these results are in strong correlation with the previous ones.

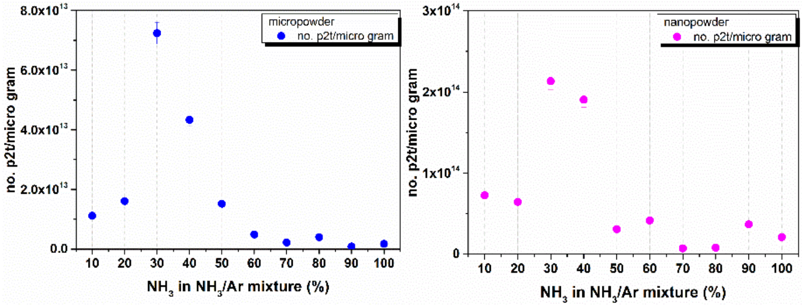

Figure 11. Quantification of the number of amine groups per microgram introduced on the surface of microsize and nanosize powder of ZnO by plasma processing.

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. 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. The outcome of this reaction can be analyzed by fluorescence microscopy as seen in Figure 12.

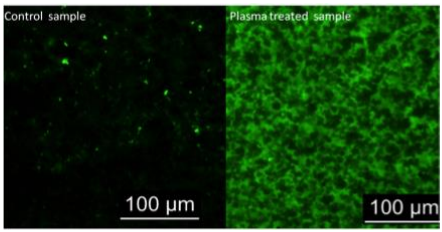

Figure 12.Fluorescene microscopy: (left) unprocessed sample and (right) plasma processed zinc oxide connected with oxidized dextran

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.

Chapter 6.Surface modification of RF magnetron sputtered zinc oxide thin films

Zinc oxide thin films were obtained by sputtering a zinc oxide target in a reactive sputtering RF magnetron sputtering system. The crystallinity and surface morphology of the as prepared samples were analyzed by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and photoluminescence measurements (PL).

Throughout our experiments, we studied the influence of plasma processing on the structural modification of the surface of the zinc oxide sputtered thin films. By changing the gas mixture to excite SWP, it was possible to enhance the properties of the materials though changing the chemical and structural composition.

Figure 13 shows the SEM images of untreated zinc oxide (as obtained by magnetron sputtering) and the images of materials processed in plasma ignited in different mixtures of NH_3:Ar. The images clearly indicate that the size of the grains of the wurtzite structure of the zinc oxide is changing after plasma irradiation. When decreasing the amount of NH_3 gas in the mixture, the surface of the zinc oxide thin films becomes much rougher, indicating that also the crystallinity of the films is changing

XRD measurements of the samples processed in all mixtures were carried out to understand how plasma processing induces changes in the crystallographic structure of the zinc oxide thin films. Figure 14 shows the same diffraction peak located at 34.46° corresponding to (002) plane and its behavior with respect to plasma treatment. It should be noticed that the intensity of this peak is increasing after processing in plasma and reaches the maximum in the case of 30%/70% NH_3/Ar gas mixture ratio used to ignite the surface wave plasma. Figure 13. ZnO SEM images of as deposited and plasma processed materials in different NH_3/Ar gas mixtures.

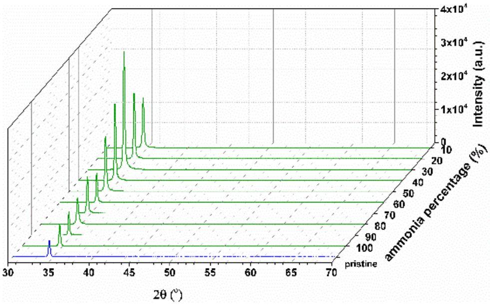

Figure 14.Comparative plotting of XRD patterns for the ZnO processed in materials in different NH_3/Ar gas mixtures.

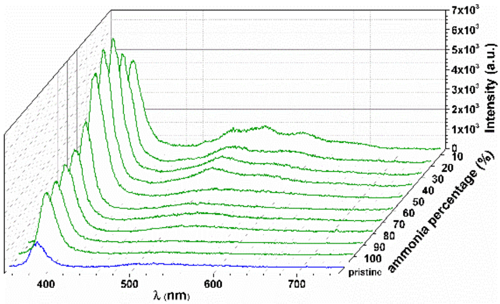

Figure 15.Comparative plotting of PL for the ZnO processed in materials in different NH_3/Ar gas mixtures.

Room temperature photoluminescence spectra of the zinc oxide were recorded and the comparative plotting is shown in Figure 15. There is an obvious change in the intensity of the photoluminescence spectra both in the ultraviolet region (corresponds to the near band gap excitonic emission) and in the visible range (green band emission). The photoluminescence results follow the same pattern like all other measurement showing the maximum intensity and enhancement when a mixture of 30% NH3 and 70% Ar is used. The results are in good agreement with the one obtained by XRD due to the enhancement of the UV emission (centered at around 380 nm) of the thin films, that usually reflects a good crystallinity of the zinc oxide. On the other hand, the green band emission can be attributed to the oxygen vacancies that where created during the activation of the surface of the thin films. It is clearly seen that by using argon in to the mixture the surface of the thin films not only it activates and easily can be grafted with amine functionalities but also changes the optical properties of the materials.

Using fluorescent dyes that specifically connect the grafted amine functionalities, it is possible to easily visualize the outcome of the plasma functionalization using fluorescence microscope. Processed samples were immersed in a dilute solution of the Alexa Fluor 488 dye to react with it. Afterwards, the samples were cleaned from excess solution and measured by fluorescence microscopy. The intensity of the fluorescence directly depends on the number of amine groups introduced on the surface of the ZnO thin films, which strongly depends on the processing parameters. Using an image processing software (ImageJ) the process can be easily quantified, as seen in Figure 16.

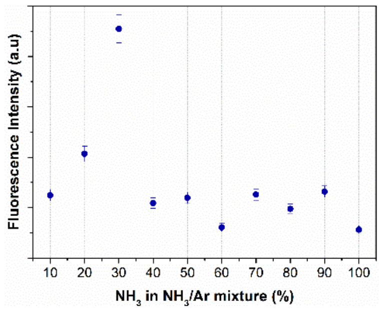

Figure 16.Comparative plotting of the fluorescence intensity of the ZnO processed in different mixtures of NH_3/Ar gases.

Chapter 7. 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 and magnetron sputtered thin films.

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.
- Crystallographic structure of the zinc oxide based nanomaterials can the enhanced by plasma processing.
- Amine functional groups can be further used as reactive sites to connect with other biomolecules.

Scientific activity

Papers supporting the thesis:

Mihai Alexandru Ciolan, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, „Mass Spectrometric Study of Ar/NH_3 Surface Wave Plasma Utilized for Surface Functionalization of ZnO Nanoparticles", Japanese Journal of Applied Physics, 53 (2014) 010207 (5pp.) (Impact Factor = 1.127)

Mihai Alexandru Ciolan, Iuliana Motrescu, Masaaki Nagatsu, Dumitru Luca, Plasma-induced functionalization of sputtered ZnO films, Matterial Letters, trimisă spre publicare decembrie 2015. (Impact Factor = 2.489)

Hu Rui, **Mihai Alexandru Ciolan**, Wang Xiangke, Nagatsu Masaaki, „Copper induced hollow carbon nanospheres by arc discharge method: controlled synthesis and formation mechanism", Nanothechnology, sent to publication 2016. (Impact Factor = 3.821)

Mihai Alexandru Ciolan, Iuliana Motrescu, Dumitru Luca, Masaaki Nagatsu, **„**Low Temperature Plasma Functionalization of ZnO with Amine Groups for Bioapplications", Digest Journal of Nanomaterials and Biostructures, Vol. 9, No. 2 (2014) p. 483 – 491. (Impact Factor = 0.945).

Awards

- 29th JSPF Annual Meeting Young Scientist Award for Plasma Applications 2012 – The Japan Society of Plasma Science and Nuclear Fusion Research,Fukuoka, Japan.
- 12th International Conference on Global Research and Education First Prize Young Scientist Award interAcademia 2013, Sofia, Bulgaria.
- Plasma Conference 2014 Young Scientist Award for Plasma Applications 2014 – The Japan Society of Plasma Science and Nuclear Fusion Research,Niigata, Japan.