Linköping Studies in Science and Technology
Dissertation No. 2083

Photoemission and Characterization of Neutrophils and Nanoparticles; Energy Mapping and Elemental Composition with sub- μm Resolution

Andreas Skallberg

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Resolution

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Cover: 3D rendering of a z-stack showing the work function distribution for two neutrophils on a silicon substrate.

During the course of the research underlying this thesis, Andreas Skallberg was enrolled in Forum Scientium, a multidisciplinary doctoral programme at Linköping University, Sweden.

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“Hälta hälta”

Abstract

Imaging and visualization of cells and tissues are important when studying various biological phenomena. The ability to provide spatial information with molecular and chemical specificity may increase our insight and understanding of biological problems within life sciences. There is a need for well suited analytical imaging tools for addressing challenges that can increase our knowledge from the visualization on the cellular and subcellular level. In this thesis, we have focused on the use of surface analytical techniques based on the photoemission process. Synchrotron based surface analytical tools such as mirror electron microscopy, low energy electron microscopy, X-ray photoelectron spectroscopy, X-ray photoemission electron microscopy and near edge X-ray absorption fine structure spectroscopy were used to obtain highly resolved chemical information for both fundamental biological systems and technical innovations.

A combined photoemission electron microscopy and imaging X-ray photoelectron spectroscopy instrument have been used for visualization and characterization of neutrophils attached to silicon and gold surfaces. Neutrophils are white blood cells and a major part of our innate immune system. In the body they circulate and scavenge for possible threats, such as pathogens. The neutrophils possess three main defense mechanisms to tackle any possible threat in the body. One of these mechanisms is the release and formation of extracellular traps used for entrapping and capturing. We have visualized the extracellular trap formation in presence of nanoparticles and images of the neutrophils have been obtained with threshold mapping and work function contrast from energy-filtering operations together with element specific imaging and chemical maps. We demonstrated work function variation in imaging mode for the cellular morphology and the characteristic polymorphonuclear morphology of the nucleus. These results demonstrate the potential and extend the use of photoemission electron microscopy and imaging X-ray photoelectron spectroscopy as analytical tools for visualization of biological materials and processes on the cellular level.

The use of nanoparticles in recent years have significantly increased. Today, nanoparticles are being used in a wide range of applications, such as in electronics, energy, biology, and medicine. One hot topic in medicine is the development of contrast enhancement agents for magnetic resonance imaging. We report the development of two types of nanoparticles to be used as contrast enhancers for magnetic resonance imaging. The first type is water-dispersible and ultra-small Fe_3O_4 nanoparticles coated with polyacrylic acid. The Fe_3O_4 nanoparticles exhibit good magnetic properties, biocompatibility, excellent relaxivity properties and can be employed as a potential dual T_1 and T_2 weighted contrast agent. The second type is cerium oxide nanoparticles with the integration of gadolinium. Cerium oxide has unique redox properties due to the coexistence of Ce^{3+} and Ce^{4+} states making them suitable for scavenging reactive oxygen species. The integration of gadolinium makes these nanoparticles promising contrast agents with both therapeutic and diagnostic properties. We have designed a new technical innovative energy saving process where a reduction in the annealing temperature for oxide removal is obtained, by the presence of europium doped gadolinium oxide nanoparticles in comparison to Eu^{3+} and Gd^{3+} . A low coverage of nanoparticles and ions revealed a significant reduction in annealing temperature for the oxide removal. These results deliver a promising one step energy saving strategy of producing silicon-based contacts.

In summary, this thesis work demonstrates the power of element specific imaging and chemical mapping of bio-related surfaces as well as nanoparticle tracking in the sub-micro and nano region.

Populärvetenskaplig sammanfattning

Avbildning av celler och vävnader är en viktig del i att studera biologiska processer. Förmågan att visualisera biologiska prover med molekylär och kemisk specificitet kan öka vår insikt och förståelse för de biologiska processerna. Idag finns det ett behov av att använda anpassade analytiska avbildningstekniker för att hantera dessa utmaningar som kan öka vår kunskap genom avbildning på den cellulära och molekylära nivån.

I detta avhandlingsarbete ligger fokus på avbildning av neutrofila granulocyter, en typ av vita blodkroppar med hjälp av ett fysikaliskt fenomen, den så kallade fotoelektriska effekten. Den fotoelektriska effekten kan förklaras genom att elektroner slås ut från ett material om man belyser det med ljus som har en tillräcklig hög frekvens. Dessa kallas då fotoemitterade elektroner. Vi har fokuserat på instrument som är baserad på den fotoelektriska effekten för visualisering och karakterisering av neutrofiler. Neutrofiler är den vanligaste celltypen av vita blodkroppar och fyller en mycket viktig funktion i det medfödda immunförsvaret. Dessa celler cirkulerar i blodet och är bland de första vid en akut inflammation. Neutrofilernas främsta uppgifter är att ta hand om och oskadliggöra främmande patogener. De använder sig av olika typer av försvarsstrategier och en av dessa består av att neutrofilen bildar och kastar ut ett nätverk som består av dess egna DNA.

Vi har specifikt använt oss av ett instrument för avbildning av neutrofiler som kombinerar teknikerna från både fotoemissionselektronmikroskopi och avbildande röntgenfotoelektronspetroskopi. Dessa instrument erbjuder ytanalyser och skapar en kraftigt förstorad bild genom de fotoemitterade elektronerna. Utöver den förstorade bilden ges spatial information om olika atomslag och den elektroniska strukturen hos de ingående molekylära komponenterna.

Dessa resultat belyser potentialen och utökar användningen av fotoemissionselektronmikroskopi och avbildande röntgenfotoelektronspetroskopi som analysverktyg för visualisering av biologiska material och processer på cellnivå.

Nanotekniken och användningen av nanopartiklar har under de senaste åren ökat markant. Nanopartiklar är små partiklar med en diameter på mindre än några miljarddelar meter. Idag används nanopartiklar i en uppsjö av olika tillämpningar, till exempel inom elektronik, energi, biologi och medicin. En utmaning idag är att tillverka och utveckla kontrastförstärkande medel baserade på nanopartiklar för användning inom bildgivande magnetresonanstomografi, en teknik för avbildning av mjuk vävnad. I denna avhandling har vi utvecklat två typer av kontrastförstärkande medel där den ena består av extremt små nanopartiklar av järnoxid som genom dess storlek kan användas som både ett positivt och negativt kontrastmedel och där den andra består av nanopartiklar som baseras på ceriumoxid med integrerat gadolinium. Dessa nanopartiklar har antioxidativa egenskaper på grund av samexistens av två oxidationstillstånd. Detta möjliggör ett kontrastmedel med både terapeutiska och diagnostiska egenskaper.

En annan tillämpning av nanopartiklar som vi har undersökt är reduktion av anningstemperaturen av kiseloxid med hjälp av närvaron av europium-dopade gadoliniumoxid-nanopartiklar. En låg täckning av nanopartiklar och joner avslöjade en

signifikant minskning av annealingtemperaturen för oxidavlägsning. Synkrotronljusbaserade tekniker har använts för att karakterisera ytan med högupplöst kemisk information. Under annealingsprocessen inträffade en fragmentering av nanopartiklarna där gadolinium migrerade till kiseloxidrika områden, medan europium migrerade till rena kiselområden. Dessa resultat visar ett lovande energisparande sätt att producera kiselbaserade kontakter.

Publication List:

Papers included in this thesis:

Paper I

Andreas Skallberg, Caroline Brommesson and Kajsa Uvdal, *Imaging XPS and photoemission electron microscopy; surface chemical mapping and blood cell visualization*, **Biointerphases Vol. 12, Issue 2, 2017**

Author's contribution: AS was the main responsible for planning, performing and evaluating the experimental work. AS prepared the gold pattern silicon surfaces and performed the stepwise surface modification. AS together with CB prepared the neutrophils and the immunoglobulin G modified silicon surface for platelet adhesion. AS wrote the main part of the paper.

Paper II

Andreas Skallberg, Kalle Bunnfors, Caroline Brommesson and Kajsa Uvdal, *Neutrophils Activated by Nanoparticles and Formation of Neutrophil Extracellular Traps: Work Function Mapping and Element Specific Imaging*, **Analytical Chemistry, Vol. 91, Issue 21, 2019**

Author's contribution: AS was responsible for planning, performing and evaluating the experimental work. AS prepared the gold patterned surfaces and the PEEM and imaging XPS characterization. AS together with KB performed the Fluorescence microscopy measurements. AS wrote the main part of the paper.

Paper III

Peter Eriksson, Alexey Tal, **Andreas Skallberg**, Caroline Brommesson, Zhang-Jun Hu, Robert Boyd, Weine Olovsson, Neal Fairley, Igor Abrikosov, Xuanjun Zhang and Kajsa Uvdal, *Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement*, **Scientific Reports, Vol. 8, Issue 1, 2018**

Author's contribution: AS was responsible of planning, performing and evaluating the NEXAFS measurements. AS contributed to the manuscript.

Paper IV

Andreas Skallberg, Kalle Bunnfors, Caroline Brommesson and Kajsa Uvdal *New Tools for Imaging Neutrophils: Work Function Mapping and Element Specific, Label Free Imaging of Cellular Structures*, **Submitted to Nano Letters**

Author's contribution: AS was the main responsible for planning, performing and evaluation of the experimental work. AS prepared the samples and conducted all the PEEM and imaging XPS measurements. AS together with KB performed the Fluorescent microscopy measurements. AS wrote the main part of the manuscript.

Paper V

Linnéa Selegård, Andreas Skallberg, Alexei Zakharov, Natalia Abrikossova and Kajsa Uvdal

Step by step Rare-Earth catalyzed SiO_x annealing and simultaneous formation of Europium-silicide by low coverage of Eu doped Gd₂O₃ nanoparticles, **Surface Science**, **2020**

Author's contribution: AS together with LS planned, performed and evaluated the experimental work; MEM, LEEM, XPS. XPEEM and NEXAFS. AS contributed to the manuscript.

Paper VI

Guannan Wang, Xuanjun Zhang, Andreas Skallberg, Yaxu Liu, Zhangjun Hu, Xifan Mei and Kajsa Uvdal, *One-step synthesis of water-dispersible ultra-small Fe₃O₄ nanoparticles as contrast agents for T1 and T2 magnetic resonance imaging*, **Nanoscale**, **Volume 6, Issue 5**, **2014**

Author's contribution: AS planned, performed and evaluated the XPS part. AS contributed to the manuscript.

Related papers, not included in thesis:

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Marian Fursatz, Mårten Skog, Petter Sivilér, Eleonor Palm, Christopher Aronsson, **Andreas Skallberg**, Grzegorz Greczynski, Hazem Khalaf, Torbjörn Bengtsson and Daniel Aili, *Functionalization of bacterial cellulose wound dressings with the antimicrobial peptide epsilon-poly-L-Lysine*, Biomedical Materials, Vol. 13, 2018

Shimelis Admassie, Anders Elfving, **Andreas Skallberg** and Olle Inganäs, *Extracting Metal Ions From Water With Redox Active Biopolymer Electrodes*, Environmental Science-Water Research and Technology, Vol. 1, 2015

Conference contributions

Oral Presentations

Blood cell visualization using PEEM and imaging XPS

Andreas Skallberg, Kalle Bunnfors, Caroline Brommesson, Kajsa Uvdal
IVC-21 NanoForum, 1-5 July 2019, Malmö, Sweden

Blood cell visualization using PEEM and imaging XPS

Andreas Skallberg, Kalle Bunnfors, Caroline Brommesson, Kajsa Uvdal
3rd Conference on Advanced Functional Materials, 21-23 August 2018, Kolmården, Sweden

Gd decorated ZnO nanoparticles to obtain high fluorescence quantum yield and enhanced MR relaxivity; potential multimodal nanoprobes for bioimaging applications

Linnéa Selegård, Maria Ahrén, Caroline Brommesson, Fredrik Söderlind, Per. O. Å Persson and Kajsa Uvdal
IVC-19, 9-13 September 2013, Paris, France

Poster Presentations

An PEEM and Imaging XPS study of Neutrophil Extracellular Traps Capturing Nanoparticles

Andreas Skallberg, Kalle Bunnfors, Caroline Brommesson, Kajsa Uvdal
AVS PacSurf Surfaces, Coatings and Interfaces Conference, 2-6 December, 2018, Hawaii, USA

Imaging and chemical state mapping of zinc oxide nanoparticles combined with gadolinium

Andreas Skallberg, Linnéa Selegård, Maria Ahrén, Caroline Brommesson, Kajsa Uvdal
IVC-19, 9-13 September 2013, Paris, France

Workshops and Meetings

XPS Imaging, 17 March 2015, Teignmouth, United Kingdom

1st NanoESCA Training School, Workshop XPS/PEEM, 8-10 October, Grenoble, France

5th FOCUS PEEM Workshop, 13-15 November, Hünstetten, Germany

CNRS THEMATIC WORKSHOP: CasaXPS Training, 8-12 July, Roscoff, France

Abbreviations

FOV	Field of View
HSA	Hemispherical Energy Analyzer
HV/UHV	High Vacuum/Ultra High Vacuum
IDEA	Imaging Double Energy Analyzer
IFMP	Inelastic Mean Free Path
LEED	Low Energy Electron Diffraction
LEEM	Low Energy Electron Microscopy
MCP	Microchannel Plate
MEM	Mirror Electron Microscopy
MOSFETS	Metal Oxide Semiconductor Field Effect Transistor
MRI	Magnetic Resonance Imaging
NETS	Neutrophil Extracellular Traps
NEXAFS	Near Edge X-ray Absorption Fine Structures Spectroscopy
PCA	Principal Component Analysis
PEEM	Photoemission Electron Microscopy
PEY	Partial Electron Yield
QDOTS	Quantum Dots
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TEY	Total Electron Yield
TOF-SIMS	Time-of-Flight Secondary Ion Mass Spectrometry
UV/VUV	Ultraviolet/Vacuum Ultraviolet
XPEEM	X-ray Photoemission Electron Microscopy
XPS	X-ray Photoelectron Spectroscopy

Preface

The time has come for me to wrap up my work in the group of molecular surface physics and nanoscience. It has been an exciting journey with a roller coaster-based progress, some ups, and some downs. I have learned a lot during my time here and I feel I have grown as a person, maybe not towards 2m in height but almost. During this journey there have been numerous people I have met that have affected me and whom I would like to reach out to with a special thank you.

Kajsa, my main supervisor, for accepting me as a PhD-student and let me become part of the NanoESCA journey that we have taken together. You have always been there for me no matter what the situation has been, both work- and personal related. Our many trips together, to Paris, Grenoble, Hawaii... have been so much fun and interesting. I still laugh when I think about that long roundish green fish that we encountered face to face during the snorkeling in Hawaii. So many memories, and I am thankful for them all, so thank you Kajsa.

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1. Introduction

Figure 1. The development of surface science with progress as a function of time. Redrawn from Höök et al. ¹

Surface science is the study of physical and chemical phenomena that are taking place at surfaces or at the interface between two phases. These phases can occur in different forms, such as solid-gas, solid-liquid, liquid-gas, and solid-vacuum. The term of a surface is referred to the outer layer of the material where the composition, chemical bonding, electronic states are often different from that of the bulk material. ² The surface science appeared in the 1960s with a focus on understanding the structure and electronic properties of clean metal surfaces, semiconductors, and adsorbates. Since late 1960s and early 1970s, the field has really diversified and developed into a broad range of scientific and technological areas. This development can be visualized by the S-curve in Figure 1, showing the progress as a function of time. The field of surface science really took off when high- and ultra-high (HV/UHV) vacuum systems were developed and subsequent when surface analytical tools emerged and became readily available to the scientific community. ¹

The development of new or improving existing surface sensitive instrumentation is a big driving force to be able to broaden the area for applications where higher spatial information within smaller dimensions are needed. One such area is focused on biological systems where imaging techniques are a vital part of studies of biological systems that can provide spatial information with molecular and chemical specificity. The ability to image and visualize cells and tissues with chemical composition on the cellular and subcellular level will broaden and increase our knowledge of biological relevant problems and biological processes. ³⁻⁷

There is a need for well suited label-free and non-destructive analytical imaging tools for imaging biological samples such as cells attached to surfaces, cellular morphologies, interactions between cells, receptors expressed at the cell surface, to address biological

processes on the cellular level.^{4, 8}

Photoemission electron microscopy (PEEM) and X-ray photoelectron spectroscopy (XPS) are two unique surface sensitive analytical instruments, based on the photoemission process, that are used for surface characterization and are today, frequently used and have shown great potential in classical surface physics and material science. Hard materials such as thin films, semiconductors and nanomaterials⁹⁻¹³ but also soft materials such as organo-materials and polymers are a few examples where PEEM and XPS characterization have shown great value.¹⁴⁻¹⁷ XPS have the possibility to identify the elemental composition and the electronic and chemical state present within a material and by extending XPS to imaging XPS, it becomes a powerful tool for obtaining the spatial distribution of the surface chemistry with fingerprint detection of elements and chemical states at sub micrometer resolution. Local variations in the electron emission are utilized in PEEM to generate images with work function contrast on the nanoscale dimension.⁴ NanoESCA is an instrument that combines these two techniques and has the potential to be extended as a label-free specific analytical imaging tool for characterization and visualization of biological samples.

Surfaces and sub-surface phenomena and nanometer sized structures have a great role in science and technological applications.¹⁸ The interdisciplinary fields of nanoscience and nanotechnology with a focus on fundamental concepts of matter in the nanoscale dimensions and the controlled manipulation of both the atomic and molecular level have led to the development of new materials with unique physiochemical properties as compared to the bulk material.¹⁹ The development of new nanomaterials is an attractive research activity and the use of nanomaterials, e.g. nanoparticles, have in recent years significantly increased.²⁰ Today, nanoparticles are being used in a wide range of applications, such as in electronics, energy, biology, and medicine due to the unique properties when scaling down the material. The development and design of nanoparticles for biomedical imaging applications, i.e. contrast enhancement agents for Magnetic Resonance Imaging (MRI) is an example of increasing research area in medicine.²¹ MRI is a powerful and indispensable tool for non-invasive real-time clinical diagnosis which delivers anatomical images of the internal structures of the body. The use of contrast enhancements is being widely used to enhance the local signal. Nanoparticles that are being used as contrast agents in MRI need to be carefully design with respect to the size, shape, structure, stability, and the biocompatibility.²²⁻²⁵

In this thesis, the focus has been on using surface analytical techniques based on the photoemission process for characterization and visualization. This can further be divided into two parts, where the first part have been directed towards biological materials i.e. neutrophils with and without interaction of nanoparticles to highlight the potential and extend the use of PEEM and imaging XPS as analytical tools for visualization of biological processes on the cellular level. The second part have been focused on the characterization and application of nanoparticles to be used as MRI contrast enhancers.

1.1 Chemical imaging of biological materials

There exists a wide range of surface analytical techniques that can be used for characterization of biological materials, surfaces, and interfaces, and a considerable number of different approaches for obtaining images of a sample, all with their own strength and weaknesses. This can involve e.g. scanning the surface using electrons with an electron beam in Scanning Electron Microscopy (SEM), using high-speed ions in Time-of-Flight Mass Spectrometry (TOF-SIMS), and X-rays in XPS, to name a few.^{6, 26, 27} Imaging and mapping elements and the chemical environment at the nano- and sub micrometer scale is an important approach and are now being used to address and provide high spatial information of biological systems and biological materials.^{3, 28}

A surface compared to the bulk material have significantly different properties in terms of composition and structure and it is at the surface where most of the reactions takes place. For example in the design of a biomaterial, it is the biological response that is mediated by the distribution of the chemical species, composition and structure of the interface that affects e.g. the protein adsorption, cell attachment and self-assembly of tissues at the surface of the material.²⁸ For biological materials such as tissues and cells where the complexity is many orders of magnitude higher than for single molecules this becomes more technically challenging. Although technically challenging, the ability to identify and image the composition and the distribution of elements at specific regions can reveal fundamental information of biological processes e.g. to be able to create elemental and chemical maps of microenvironments such as tumors, could benefit from these types of visualizations. Also, the knowledge about chemical changes due to biological stresses within tissues and cells could be increased by probing the environment with molecular and chemical specificity.⁸ When analyzing biological materials using surface analytical techniques, it is of main importance to be aware of that most of the instrument work under ultra-high vacuum, where careful sample preparation and handling is crucial.²⁸

The ability for chemical imaging to visualize the distribution of metals such as zinc, copper and iron, to name a few, which play a fundamental role of a cells biochemical functions and elements such as nitrogen, phosphor and sulfur that are part of the building blocks of biomolecules can bring information of the cells function and physiology in response to several conditions. Also, elemental mapping of the distribution of metal-based nanoparticles for e.g. tracking their fate in a biological context will help assessing its impact and toxicity.²⁹⁻³¹

This challenge in analyzing biological materials is a driving force for developing or improving existing surface analytical instruments and recent technological progress have made it possible for chemical imaging with increased spatial resolution and improved sensitivity.²⁹ For surface characterization, each surface analytical technique provides with unique information, and for obtaining as much data as possible, it is often necessary to use multiple techniques. By using both PEEM and imaging XPS on the same sample and well-defined area, a more comprehensive view of the sample can be obtained. The combination of the two will broaden the perspective and contributing to the big picture.

1.2 Nanoscience/Nanotechnology/Nanoparticles

Nanoscience is a cross disciplinary field where fundamental concepts of matter that have nanoscale dimensions are being studied. Nanotechnology on the other hand is the controlled manipulation of both the atomic and molecular level to develop, design and produce new materials with unique properties that are different from the bulk material. The concept was first described in 1959 by Richard Feynman.³²

Nanomaterials are in general categorized as materials that have at least one dimension in the nanoscale i.e. less than or equal to 100 nm with unique physical, chemical, mechanical, and optical properties different from that of the bulk material.^{19, 33} Nanomaterials are a bridge between bulk materials and atoms and molecules and depending on the material and the structured components, nanomaterials can be further classified as one-dimensional (one dimension inside the nanoscale i.e. thin films), two-dimensional (two dimensions inside the nanoscale i.e. nanowires, nanorods and nanotubes) and three-dimensional (all three dimensions exist in the nanoscale i.e. nanoparticles).³⁴

The use of nanoparticles e.g. polymers, metals and metal oxides have in recent years significantly increased and these nanoparticles are used in a wide range of applications such as sensing, signaling and chemical detection to name a few.³⁵⁻³⁷ An area within medicine where nanoparticles are of increasing interest is biomedical imaging applications, i.e. contrast agents for signal enhancement in MRI.³⁸⁻⁴⁰ Today, there is a need to design nanoparticles to identify specific diseases at the tissue and cell level. The effect of scaling down a material to the nanometer range will bring forward unique physiochemical properties which makes nanoparticles suitable for biomedical imaging. This includes a large surface to volume ratio, high reactivity with many atoms located at the surface and the ability for surface modification.⁴¹ Through the surface modification it is possible to tune the blood circulation time, diffusivity and decrease the immunogenicity.^{42, 43}

One other direct effect when scaling down the material is the appearance of a quantization effect. This is related to the confinement and the movement of the electrons to discrete energy levels and depending on the size of the nanoparticles makes it possible to tune the magnetic, electrical, and optical properties.³³

1.2.1 Rare-earth elements

Seventeen rare-earth elements exist in the periodic system where fifteen of them (La 57 – Lu 71) are lanthanides as well as scandium (Sc 21) and yttrium (Y 39). Although Sc 21 and Y 39 are not lanthanides, they are considered as rare-earth elements due to that they exist in the same ore deposits. Rare-earth elements exhibit unique electronic, optical, and magnetic properties owing to their unpaired 4f electrons.^{44, 45} There exist relatively much rare-earth elements in the minerals despite the name. The name rare-earth comes from that they are usually dispersed and found in low concentrations. Cerium, gadolinium, europium, and yttrium are rare-earth elements that have been used for developing nanoparticles to be used as contrast agents in MRI or as components in Quantum dots (QDots), which are further discussed below.

1.2.2 Gadolinium integrated cerium oxide nanoparticles

Cerium (Ce) is rare-earth elements and one of the lanthanides with the atomic number 58. Cerium oxide has been shown to possess antioxidant behavior through its unique redox properties due to the coexistence of Ce^{3+} and Ce^{4+} states.⁴⁶⁻⁴⁸ These redox properties have been shown to be able to scavenge reactive oxygen species (ROS). ROS are unstable molecules of reduced oxygen, e.g. hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) and generated as a natural byproduct from normal cellular metabolism with a potential of damaging DNA, proteins, and lipids.⁴⁹ Depending on the environment e.g. in presence of ROS, the oxidation states of cerium can be switched and function as a regenerative antioxidant.⁵⁰

Gadolinium (Gd), also a lanthanide and one of the rare earth elements but with the atomic number 64. Gd has a large magnetic moment because of its seven unpaired f-electrons and at room temperature it shows paramagnetic properties, i.e. magnetic properties where the spin can align with an externally applied magnetic field.⁵¹ Together with the slow relaxation rate due to the symmetric electronic structure makes gadolinium a commonly used contrast agent for MRI.⁵²

The combination of a cerium oxide core with integration of gadolinium have been developed and characterized in **Paper III**, as promising contrast agents with both therapeutic and diagnostic properties.

1.2.3 Ultra-small iron oxide nanoparticles

Metal oxide nanoparticles of iron oxide have been shown as a promising material for contrast enhancement in MRI due to low toxicity and the magnetic properties.⁵³ Contrast agents based on iron oxide were first considered as T_2 -agents and delivers a reduced signal in the MR image.⁵³⁻⁵⁵ Iron oxide nanoparticles with extremely low dimensions, i.e. ultra-small iron oxide nanoparticles have recently gained attention to be used as a T_1 -agents, which enhances the signal and increases the local contrast. Ultra-small iron oxide nanoparticles show superparamagnetic features due to the extremely small size and in **Paper VI**, we have synthesized, water-dispersible and uniform ultra-small Fe_3O_4 nanoparticles as a potential T_1 and T_2 dual contrast enhancement agent in MRI.

1.2.4 Europium doped gadolinium oxide nanoparticles

In opto- and microelectronics, there is a challenge to find appropriate contact and interconnect materials as the scale of an electronic device is getting smaller and smaller.⁵⁶ Today, there is a growing interest to produce nanoscale building blocks based on metal silicide as integral parts of these devices. The use of rare-earth elements e.g. europium (Eu 63), in combination

with silicon, to produce europium silicide provides a material that can be used in metal-oxide semiconductor field effect transistors (MOSFETS), where the europium silicide can function as ohmic and Schottky barrier contacts with properties such as high channel mobility, small junction depth and low resistance.⁵⁶⁻⁵⁸ The production of rare-earth silicide is usually carried out by epitaxial growth, where the rare-earth material is deposited onto pure and clean silicon substrate followed by annealing.⁵⁹ The need for pure and clean silicon without any oxide is crucial to produce a junction as smooth as possible. The process for oxide removal goes through a series of annealing steps and temperatures where typically inhomogeneous decomposition and formation of voids appear at about 900-1050°C.⁶⁰ Since a reduction in this annealing temperature is highly appreciated, we have in **Paper V**, presented the study of the annealing temperature of oxide removal and subsequent formation of europium silicide by deposition of Eu-doped gadolinium oxide nanoparticles onto silicon oxide substrates.

1.2.5 Quantum dots

Qdots are semiconducting nanocrystals with tunable optical and electrical properties. The band structure in a semiconducting bulk material consists of a valence band and a conducting band. Upon addition of energy e.g. irradiation of light, electrons can jump from the valence band to the conduction band thus making the material conducting.⁶¹ In a semiconducting bulk material, the energy band is homogeneously distributed, but when scaling down the material and with the quantization effect, a quantum confinement of the electrons and holes, discrete energy levels appears with a wider band gap between the valence- and the conduction band. The effective band gap will increase with decreasing crystal size. This in turn affect the optical properties, with emission of fluorescence light at higher energies with decreasing size.⁶² The composition and size-dependent emission and absorption properties, photostability and fluorescent lifetime of QDots, makes them great agents for bioimaging in both in vitro and in vivo studies.^{61, 62}

In **Paper II** we have used amino-poly(ethylene glycol) (NH₂-PEG) functionalized cadmium selenide (CdSe) zinc sulfide (ZnS) QDots as a marker for labeling and neutrophil interaction and the release of neutrophil extracellular traps (NETs). The QDots also allows for comparison between PEEM and imaging XPS and Fluorescence microscopy.

QDots have proven valuable as building blocks for the development of biosensors. The development of biosensor with high sensitivity and selectivity is of great interest for both fundamental biomedical research both also within clinical diagnostics. QDots electrical and optical properties makes them suitable as building blocks for the development of biosensors. The application area of a biosensor with QDots incorporated have shown valuable for the detection of DNA, proteins, enzymes and for cells.⁶³

In **Paper I** we have used europium doped yttrium vanadate (Eu-YVA) QDots for specific attachment to a self-assembled monolayer on a gold patterned silicon substrate to show the potential of elemental imaging using PEEM and imaging XPS.

1.3 Magnetic resonance imaging (MRI)

MRI is a non-invasive, powerful diagnostic technique used for mapping of the internal structures of the body. The technique is today, widely used at clinics throughout the world for routinely acquiring detailed information of the anatomy of organs and soft tissues.⁶⁴ MRI was developed by Sir Peter Mansfield and Paul Lauterbur which earned them the Nobel prize in medicine in 2003.⁶⁵

Magnetic resonance is based on the phenomena that when a nucleus of odd number of protons of a certain atom possess a net charge, can absorb, or emit energy when placed in a magnetic field. The most common nucleus which possess a net charge in all organisms is the hydrogen nuclei thus making MRI a powerful imaging technique for mapping soft tissues of the body.⁶⁶ Hydrogen and other atoms with a net charge possess an angular momentum called spin. The magnitude of the spin is depended on the spin quantum number I , which is related to the inner structure where the angular momentum has spin directions equal to $2I+1$. Hydrogen has spin equal $\frac{1}{2}$ and can thus adopt two spin directions. In MRI, an external magnetic field is applied, and the spins will align parallel or antiparallel to the magnetic field and a net magnetization is created. By applying radiofrequency pulses, a change in the spin direction and net magnetization occurs and after a short time a relaxation in the spins towards equilibrium will be recovered. The spin relaxation of hydrogen protons induces a small voltage that being collected for creating the image, and it is the relaxation time that being probed in MRI.^{67, 68} To improve the contrast in MR images and to further differentiate diseased tissues from healthy, contrast agents are being used. A contrast agent shortens the relaxation time for the protons to realign with the external magnetic field. Contrast agents are usually divided into positive (T_1) and negative (T_2) contrast enhancers, where a T_1 contrast agent enhances the signal in the MR image, while a T_2 agent, reduces the signal.⁵⁴

The first clinically used contrast agent was based on gadolinium, namely gadopentetate dimeglumine (Magnevist)²⁰ and based on chelates where metal ions are coordinated into organic matrixes. Contrast agents based on nanoparticles can contain more ions per particle which will further enhance the contrast in the image.

1.4 Neutrophil granulocytes

Neutrophils are the most abundant type of leukocyte and a predominant part of the innate immune system. Neutrophils are quick responders to a microbial threat and one of the first to migrate to a site of inflammation.⁶⁹ They are produced in the bone marrow and in a normal adult roughly 100 billion neutrophils are produced daily. Once present in the blood stream, they circulate in great numbers with a fairly constant concentration of $\sim 3\text{-}6 \times 10^6$ cells/ml in a healthy human with a life span of a few hours before they undergo spontaneous apoptosis and cleared away by macrophages.⁷⁰

Neutrophils are also polymorphonuclear cells meaning that they have two distinct morphological characteristics, a multilobulated and condensed nucleus and multiple intracellular granules. Granules are small vesicles that contains e.g. specific loads of acid hydrolases and antimicrobial peptides.^{71, 72}

Neutrophils possess three main strategies, both intra- and extracellular, for neutralizing of invading microbial threats, e.g. pathogens.⁷³

The first strategy for fighting invaders is phagocytosis, where the threat is engulfed into a phagosome, combined with the lysosome and killed by ROS. The second strategy is by degranulation, where ROS and antimicrobial peptides are released to the extracellular matrix as a response to the detected threat. The third main strategy is the release of NETs, a network which is composed of DNA, histones, proteins, and ROS.

The capability to rapidly change shape to pass through a narrow capillary and to spread out and adhere to e.g. foreign material or intruder is one of the most important abilities of the neutrophils and is correlated to the activation process. A surface physical, chemical and topographical properties all affect the adhesion and the spreading of neutrophils and once a neutrophil adhere and spread onto a surface their membrane can expand to twice its size. During cell spreading, neutrophil form pseudopods, arm-like structures consisting of cytoplasm and actin filaments. Detailed characterization using elemental and chemical imaging for changes in cellular morphology and cell-surface interaction are of importance for understanding spreading of neutrophils and to modify surfaces for e.g. non-specific cell adhesion.⁷⁴⁻⁷⁷

The use of neutrophil as a model system for e.g. biocompatibility of nanoparticles, is a great way of studying cellular processes and ROS production. In this thesis, neutrophils attached to silicon and gold surfaces have been used as model system for imaging using the combined PEEM and imaging XPS instrument with and without interaction of nanoparticles (**Paper I, II and IV**) and as an antioxidant assay for ROS production measurements (**Paper III**).

1.5 Neutrophil extracellular traps (NETs)

NETs are one of the main strategies in the host defense response with the release of extracellular traps. NETs were first discovered in 1996 by Takei et al.⁷⁸ and further explained by Brinkmann et al.⁷⁹ in 2004 and has since then gathered a lot of interest. NETs are also considered to be a key component in certain pathological conditions such as autoimmune diseases, diabetes, and cancer.^{80, 81}

NETs are abundant at sites of inflammation and the release of NETs is an active process called NETosis. It is a type of cell death that is different from apoptosis and necrosis. Apoptosis is a programmed natural cell death and degradation with the purpose of recycling cytoplasmic components, whereas necrosis is a traumatic cell death characterized by cytoplasmic swelling with disorganized organelles and ruptured plasma membrane.⁸² Although NETs have been discovered over 20 years ago, the detailed mechanistic picture and the specific signaling that leads to NETs formation are still largely unclear and under investigation.⁸¹

There are several roles of NETs in infections. NETs can immobilize microbes by trapping and function as barriers to minimize the spreading. The components of NETs, i.e. DNA and proteins can function as immune signaling to other cells to help the inflammatory response. One example of inflammatory response is from macrophages where they sense these immune signals and in turn produce proinflammatory mediators. At high concentrations, NETs can resolve an inflammation by cleaving of proinflammatory cytokines.^{81, 83} It has been shown that NETs can be triggered by small molecules (e.g. phorbol 12-myristate 13-acetate, PMA), nanoparticles, bacteria, virus, fungi and parasites.⁸³

The process of NETosis can be divided into two main paths, “vital NETosis” and “suicidal NETosis”.^{84, 85} The general mechanism for both paths is characterized by the disintegration of the nuclear membranes and a decondensation of chromatin. For “suicidal NETosis”, the decondensed chromatin is mixed with cytosolic and granule proteins which fills up the neutrophil until the cell membrane ruptures and NETs are released in the extracellular space as a web-like structure with fibers of chromatin decorated with intracellular components, e.g. antimicrobial peptides and proteins.^{71, 79, 86}

The “vital NETosis” path is a non-lytic form which involves the release of NETs through degranulation without rupturing the cell membrane.^{84, 85} A schematic overview of NETs formation is shown in Figure 2.

Figure 2. Illustration of NETs formation. A neutrophil in a resting form with a round shape and segregated nucleus starts to upon activation disintegrate the nuclear membranes. Decondensation of chromatin is starting and the integrity of the chromatin is gradually lost. Granular and cytoplasmic components is mixing with the decondensed chromatin until it fills up most of the cell up until the cell membrane ruptures and subsequent the release of NETs. Copyright 2020 American Vacuum Society.

1.6 Photoemission

The photoemission process is one of the fundamental importance for many of the surface characterization instruments. Photoemission is the process where electromagnetic radiation is absorbed and consequently emission of electrons. This process was observed by Hertz ^{87, 88} in 1887 but it was not until 1905 when Einstein ⁸⁹ explained this process, which awarded him with the Nobel Prize in Physics in 1921. ⁹⁰ Einstein introduced the concept of photons and a relationship between the photon energy ($h\nu$) and the kinetic energy of the emitted electrons (E_{kin}) which led to the fundamental photoelectric equation:

Equation 1.
$$E_{kin} = h\nu - \phi,$$

where ϕ is the work function and defined as the minimum energy needed for an electron to be removed from the fermi level into the vacuum.

The photoemitted electrons contains information from the photoelectric yield, the distribution of kinetic energies, the emission angles, and the positions from where they left the sample surface. The photoemission process can be described using a three-step model that includes photoexcitation of electrons, electron propagation inside the material to the surface and transmission of electrons through the surface into the vacuum and are further explained in the following sections.

Figure 3. Schematic representation of photoemission. Photons are incident on the sample and subsequent emission of electrons.

1.6.1 Photoexcitation

The first step in the three-step model is the photoexcitation of electrons. The sample is irradiated with photons and depending on the ionization energy of the incoming photons, secondary-, valence- and/or core level electrons are being excited. The photons penetrate through the sample until they are absorbed or until the energy is lost by electron excitations. For all elements there exist a probability for photon absorption. This probability is described by the photoionization cross section and it depends on the energy of the incoming photons and the elements that the photons are propagating through. ⁹¹ The photon absorption dependence

of the energy of the incoming photons, makes it possible to tune the photon energy over a specific XPS peak, to enhance the signal. This is demonstrated in Figure 4.

Figure 4. Photoionization cross section of three selected peaks, Gd3d, Gd4f and Gd4p, to demonstrate the photon absorption dependance with the photon energy.⁹²

1.6.2 Propagation

The second step in the three-step model is the transport and propagation of electrons to the surface. Inside the material, the excited electrons travel in all directions where they suffer elastically and inelastically scattering processes. The main scattering process of the electrons is electron-electron interactions but is also dependent on crystal defects.⁹³ This process will lead to a loss in energy with a reduced number of photoexcited electrons that can reach the surface and will contribute to a broad continuum background of secondary electrons in the final spectrum. In a photoemission experiment using high energy light sources, most of the emitted electrons are secondary electrons. When an electron is excited to a higher energy state, this primary electron can exchange energy and momentum with other electrons inside of the material. This exchange also promotes the electrons to higher energy states which will lead to that from absorption of a single photon, more than one electron can be emitted out from the material.

The effect of inelastic scattering process is commonly described by how much the photoelectron signal intensity is decaying along the traveled path:

Equation 2.
$$I(l) = I_0 e^{\left(-\frac{l}{\lambda_{in}}\right)}$$

where I_0 is the original intensity of the electrons, l the path distance and λ_{in} is the inelastic mean free path (IMFP). The IMFP can be described as the average distance an electron can travel without being inelastically scattered.⁹⁴ The IMFP is dependent on the kinetic energy of the electrons and the quantity follow a similar behavior in many materials, which is illustrated by the so-called universal curve of the energy dependence of the IMFP, shown in Figure 5.

Figure 5. The universal curve for inelastic mean free path of electrons.

1.6.3 Transmission

The last step in the three-step model is the transmission through the surface. In order for the photoexcited electrons to transmit through the sample surface, the kinetic energy of the electrons must overcome the surface potential barrier, otherwise the electrons will be reflected back.⁹³ The cut-off energy where the electrons have insufficient energy to be transmitted through the sample surface is the work function and by measuring this cut off energy provides a convenient way of determining the work function of the sample.

Figure 6. A schematic representation of the photoemission process described by the three-step model. 1. Incoming photons excites electrons (core level and valance electrons) within the sample. 2. The excited electrons propagated through the sample. While propagating through the sample, the excited electrons interact with other electrons causing emission of low energetic secondary electrons. 3. The excited electrons can be transmitted through the sample surface to reach the detector if the energy of the electrons is higher than the sample work function. Figure inspired by Hüfner ⁹³.

2. Methods

2.1 Photoemission electron microscopy (PEEM)

PEEM was first realized by Bruche in 1933 when he showed that it was possible to use a simple magnetic lens to form an image of electrons photoemitted from a solid surface by irradiation of ultraviolet light.⁹⁵ This marked the beginning of surface imaging with photoelectrons using immersion lenses, and since then, PEEM have shown great potential and found application in a broad range of different research fields, e.g., material science, surface physics, surface chemistry etc.^{13, 96}

PEEM is a versatile instrument since it can be combined with a range of light sources thus obtaining information about the structural, chemical, electronic, and optical properties of surfaces and interfaces.⁹⁷ PEEM belongs to the family of cathode lenses and images are obtained by irradiating the sample with photons. Electrons are emitted from the sample and accelerated by strong electric fields inside an objective lens. High magnified images of the sample are then produced by the objective and the photoemitted electrons are projected onto a multichannel plate intensifier where they are amplified and imaged on a phosphorus screen. A CCD camera is then used to capture that data from the phosphorous screen.^{98, 99}

The use of photoemission for imaging biological materials was first introduced when O.H Griffith et al¹⁰⁰ publish photoelectron images on thin sections of rat epididymis. They demonstrated the possibility to image a biological surface where the exposed molecules exhibited a difference in the ionization potential. Images of rat epididymis with good contrast were achieved, although the initial magnification was rather low. PEEM as an electron-optic analog to fluorescence microscopy and they used it for imaging of biological samples such as cells, viruses, and DNA, with both non-labeled and labeled gold and silver particles to show the capabilities of biological focused PEEM.¹⁰¹

The use of synchrotron-based X-ray photoemission electron microscopy (XPEEM), makes it possible to characterize and image surfaces at even higher lateral resolution, down to a few tens of nanometers. The unique characteristics of the light that is produced at a synchrotron storage ring have a high flux and brilliance; many orders of magnitude higher than light produced with a conventional source. The synchrotron light is also highly polarized, collimated, and tunable, which makes it possible to choose energy for the outcoming light. This is particularly useful since it overcomes the limited elemental sensitivity from a conventional light source.^{97, 102}

The contrast generated in an image from a PEEM measurement depends on what kind of light source being used for irradiation and on the sample itself. On rough samples, topographical contrast in the image will appear due to the curvature of the accelerating field around these features. For work function contrast, an ultraviolet (UV) light source with an energy close to the photoemission threshold is usually being used, where differences in the electron emission generate differences in the local work function. Elemental and chemical contrast are generated using higher energy light sources, e.g. X-rays by acquiring core level electrons over a specific element. The contrast when obtaining images with X-rays is lower compared to images from

UV light due to the fact that there is a much larger energy spread of the electrons emitted using X-rays, which then will be dominated by chromatic aberrations.⁹⁷ Although at a synchrotron facility, where the possibility to adjust the photon energy for a specific element or over an absorption edge, the contrast for core level electrons will increase.

2.2 X-ray Photoelectron Spectroscopy (XPS)

XPS was developed in the 1950's by Kai Siegbahn and co-workers as an analysis tool where he developed a method that could achieve spectra with discrete lines that corresponds to different energy levels in atoms. He also showed that the binding energy of the photoemitted electrons could reveal the local chemical environment with chemical shifts.¹⁰³ For this work, Siegbahn received the Nobel Prize in physics 1981.⁶⁵ XPS is an established and frequently used technique for understanding the nature of a sample surface. In surface analysis, XPS has over the past 30 years become the most used technique and has expanded over many research areas from classical surface physics and materials science towards environmental and biological systems.¹⁰⁴

XPS is a surface sensitive analytical technique used to identify and quantify the elements present in a sample. XPS can be used for obtaining information on the elemental composition and the chemical and electronic states of each element, except for hydrogen and helium.¹⁰⁵ XPS can also be used as angle-resolved by vary the emission angle of the incoming light, from an angle normal to the surface towards a near-grazing angle, thereby detecting photoemitted electrons at varying depths. This is particularly useful when characterizing ordering of layers and for determining the thickness.^{106, 107}

The technique is based on the photoelectric effect, where high energy photons ($h\nu$) are incident on the sample. Emission of photoemitted electrons will occur if the energy of the incoming photons is greater than the binding energy (E_b) and the work function (ϕ) together. The kinetic energy (E_k) of photoemitted electrons are measured by the detector and by using equation 3, the binding energy can be calculated.¹⁰⁸

Equation 3.
$$h\nu = E_k + E_b + \phi$$

Core level electrons and the binding energies of the photoemitted electrons are unique and will be a fingerprint of the elements present in the sample. Core level electrons are also sensitive to the chemical environment thus chemical information of chemical shifts i.e. change in the local bonding environment can be obtained with XPS.

The surface sensitivity and the analysis depth of XPS is based on those electrons which has escaped the sample surface into the vacuum and reached the detector. This is related to the IMFP of the electrons i.e. the distance an electron can travel without being inelastically scattered and losing the energy.¹⁰⁹

The IMFP, the flux of the photons, the number of atoms per unit area and the photoionization cross section contributes to the total intensity in XPS. The photoionization cross section is the

probability of an electron being emitted from a certain electronic state and is unique for every single element and electronic state. For elemental quantification in XPS, the relative intensities are used to calculate the relative concentration for each element present. The relative ratios of the elements present are divided by the specific photoionization cross section and is calculated using:

Equation 4.
$$n_a = \frac{I_a/S_a}{I_a/S_a + I_b/S_b + \dots + I_n/S_n}$$

where n_a is the relative concentration, I_a is the intensity of the emitted electrons and S_a is the atomic sensitivity factor.

XPS measurements have proven valuable in the analysis of chemical and structural properties of complex biological samples, such as bacteria, diatoms, fungi, and yeast.^{26, 110-112} For these samples the use of XPS was focused on cell surface properties and chemical changes in the cell wall compositions and how it changes for a variety of parameters including the surface charge, pH variations and functional groups.

The characterization of surfaces and interfaces with local chemical composition, molecular structures and distribution of chemical species is crucial to be determined since the adsorption of proteins and attachment of cells are depending on the presence of biological and chemical moieties of the surface. XPS can and have shown great value in characterization of adsorbed proteins, enzymes, lipids, and DNA onto surfaces for e.g. the development of biosensors and bio arrays by obtaining information on the chemical functionality.^{2, 113}

The advancement in developing and improving new and existing instruments will increase the ability for high spatial resolution where laterally resolved elemental and chemical maps are needed to provide detailed information.^{2, 113}

2.3 NanoESCA

The main instrument for surface characterization in this thesis have been by using an instrument called NanoESCA. This is an instrument that combines the techniques of PEEM and imaging XPS and it is based on an electrostatic photoemission electron microscope with the addition of an imaging double hemispherical energy analyzer (IDEA). The IDEA is arranged so that they are rotated 180° towards each other and coupled with a transfer lens. The concept of the IDEA is due to that in a spherical electrostatic field, the electron trajectories will coincide after a full 360° orbit, independent of the starting energy and emission angle. This results in an image without any achromatic aberrations. The IDEA arrangement will enable a complete orbit of the electron trajectories and compensate for chromatic and spherical aberrations. This setup eliminating the energy spread and the final resolution will only be limited by the objective lens aberrations.¹¹⁴⁻¹¹⁶ A schematic illustration of the IDEA arrangement and the electron trajectories are shown in Figure 7.

Figure 7. Schematic illustration of the electron trajectories in a hemispherical energy analyzer (HSA), a spherical orbit and the optical layout for the IDEA arrangement. The largest dispersion α^2 , is introduced after a 180° orbit of the electrons, which is induced through a normal XPS HSA. In a spherical setup, the electrons will coincide after a full 360° orbit independent of the starting energy and emission angle. This is used for the NanoESCA, where the second energy analyzer is rotated 180° towards the first energy analyzer, cancelling out the aberrations. Redrawn from¹¹⁵

2.3.1 Energy and lateral resolution

The lateral resolution of the instrument i.e. about 40 nm and 500 nm for low energetic secondary electrons and core level electrons, respectively is achieved by the immersion objective lens that uses high voltages (12 kV) for extraction of the photoemitted electrons. The PEEM column acts as an entrance lens for the energy analyzer where a drift potential of about 1000 V is applied. The electrons are decelerated in the focusing field on the drift potential by asymmetric voltages from the objective lens. For high transmission operation

with small aberrations, the low PEEM column potential is beneficial for the retardation of the electrons towards low pass energies. This render in the advantage of spectroscopic imaging and still maintaining the advantage of a high accelerating field.¹¹⁴

Within the PEEM column, iris- and contrast apertures are located for defining specific regions of interest inside of the field of view (FoV) and also to increase the contrast by minimizing the spherical aberrations by restricting the angular acceptance.¹¹⁴

Figure 8. Schematic illustration of the NanoESCA instrument. Redrawn from¹¹⁵

2.3.2 Excitation sources

The NanoESCA is equipped with three excitation sources.

- 1) UV mercury (Hg) discharge lamp with an energy of 5.2 eV.
- 2) Vacuum ultraviolet (VUV) helium I (He) discharge lamp providing an energy of 21.2 eV.
- 3) Monochromatized Al K α X-rays with photon energies of 1486.6 eV.

Photons are incident on the sample at an angle of 65° with respect to the surface normal and

images of the photoemitted electrons are captured by the microscope. Information are obtained from low energetic secondary electrons and valance electrons with work function contrast and core level electrons with elemental and chemical contrast.

2.3.3 Operation modes

Three modes of operation exist for the NanoESCA,

- 1) Direct non-energy filtering imaging.
- 2) Energy-filtering imaging.
- 3) Small spot, area selected spectroscopy.

For direct non-energy filtering imaging operation, the electrons are magnified onto an image intensifier without passing through the energy analyzers. The image is formed with the entire set of photoelectron energies. This render in information from secondary electrons and is related to the entire photo-yield.

During energy-filtering PEEM operation, the electrons are focused to the entrance slit of the first hemispherical energy analyzer through the entrance lens system. The photoemitted electrons are decelerated from the drift potential to the set pass energy of the first energy analyzer.

Small spot, selected area spectroscopy mode makes it possible to choose an area of interest by using an adjustable iris aperture that is in the back focal plane of the first intermediate image plane. Photoemitted core level or valance electrons are collected using a channeltron detector after the first hemispherical energy analyzer.

2.3.4 Energy-filtering and work function maps

During NanoESCA energy-filtered mode operations, the measurements are done with a full field parallel imaging mode. This allows for selecting regions of the photoemission spectrum, from low energetic secondary electrons, valance electrons to core level electrons, where the energy axis is scanned. This produces three dimensional image stacks where the spatial contrast will be a function of the photoelectron kinetic energy $I(x,y,E-E_F)$, with spectroscopic and microscopic information in every image pixel.^{117, 118}

Figure 9. Energy filtered image series of an Au/Si interface. The image stack has been obtained from $E - E_F = 2.0 - 5.5$ eV in steps of 0.05 eV. Acquiring energy-filtered images produces three-dimensional image stacks, where every image corresponds to a single energy.

To generate local work function maps, images over the photoemission threshold are acquired with increasing photoelectron kinetic energy ($E - E_F$) which produces three dimensional image stacks with intensity $I(x, y, E - E_F)$. Local work function values are calculated for each pixel $I(x, y)$ by fitting the photoemission threshold $I(E - E_F)$ using an complementary error function:

Equation 5.
$$I(E - E_F) = \frac{I_{max}}{2} \operatorname{erfc} \left(\frac{\phi - (E - E_F)}{\sigma\sqrt{2}} \right) + I_{off}$$

where I_{max} is the maximum intensity of the spectrum of each pixel, I_{off} corresponds to the intensity offset, ϕ local work function and σ is the standard deviation of the gaussian. This factor is pass energy dependent and due to the broadening of the image spectrometer. The obtained work function values are then used to create two-dimensional-maps of the work function distribution that being acquired in each pixel.¹¹⁹⁻¹²¹

2.3.5 Instrumental induced artefacts

Prior to analysis, instrumental dependent artefacts superimposed by the microchannel plate (MCP) and the CCD camera need to be corrected for. These corrections and filtering are all made post measurement. Effects that are usually corrected for is the non-isochromatic along the vertical axis, the Schottky effect, dark- and flat field corrections.

The non-isochromatic along the vertical axis in the image is due to an energy shift within the images and corresponds to the dispersive plane of the image spectrometer. Although the correction of the α^2 aberration, the IDEA introduces non-isochromaticity in the images. This means that for different points along the y-axis represent different energy values.¹²² This energy shift is proportional to the pass energy of the analyzer and for a single point x_0 in the image, the corresponding energy shift can be calculated by:

Equation 6.
$$\Delta E = E_{pass} \left(\frac{M_1 x_0}{f} \right)^2$$

where M_1 is the lateral magnification of the microscope in the first image plane and f is the focal length of the entrance lens. This energy shift is corrected for in energy-filtered mode operations.

Due to high electric fields and high extraction voltage between the sample and the extractor a Schottky effect¹²³ appears which will lower the value of the work function according to the equation:

Equation 7.
$$\Delta E_{Schottky} = -e \left(\frac{e E_{ext}}{4\pi\epsilon_0} \right)^{1/2}$$

where e is the elementary charge, E_{ext} is the electric field for the extractor and ϵ_0 is the vacuum permittivity ($\epsilon_0 = 8.854188 \times 10^{-12} \text{ F m}^{-1}$). During an experiment, the extractor voltage is set to 12 kV with a distance between the sample and the extractor being 1.8 mm. This results in a $E_{ext} = 6.66 \times 10^3 \text{ kV m}^{-1}$ and consequently a lowering of the work function of 0.098 eV. This effect is then being corrected for.

During measurements with the NanoESCA, especially for energy-filtered operations with core level electrons, the photoelectron signal is quite weak. This means that longer acquisition times are needed and will induce bright pixels in the image that does not corresponds to any real information from the photoelectron signal. To adjust for these dark fields, an image without any photoelectron signal is acquired and then subtracted with the original image. The acquisition time for the real data measurement and the dark field image must be the same. The MCP consists of a hexagonal pattern and this is imposed onto the real measurement images. There can also be some small dust particles between the camera and the screen. All these are corrected for by a flat field image i.e. an image obtained over a homogenous area of the sample and then divided the original images with that flat field image.

2.3.6 Data processing

In imaging XPS, longer period of acquisition times, typically hours, are being used to extract core level electrons. This render in high intensity pixels and noise to the image-data set. After scanning the kinetic energy over a specific element, the need for data reduction to increase the signal-to-noise ratio and to filter out high intensity pixels are necessary. High intensity pixels randomly distributed over the individual images are first filtered out before by using an outlier filter. The outlier filter computes and compares one pixel intensity with surrounding pixel intensities and by a determining a threshold for the pixel intensity, the outlier filter can replace the individual pixel with a mean value of the surrounding pixel intensity if the intensity is within the set threshold. This generates images with good pixel intensities for further processing and analysis.^{5, 124, 125}

Every pixel in the image contains a spectrum and these spectra are used for data processing. Multivariate techniques such as principal component analysis (PCA) have been used in this thesis to create enhanced images with a lower content of noise. In PCA, a set of orthogonal images are being created where the maximum variation is partitioned into separate abstract factor images that contains the largest eigenvalues. Those abstract factor images that contains no features can be attributed to noise and by using the abstract factor images with significant features, which have the greatest variance, to reconstruct the entire image-data set, a new image-data set will be generated with smoothing in the spatial domain and with reduced noise.^{124, 125}

After data reduction procedures, pixel-by-pixel analysis can be made by measuring the photoelectron peak areas and normalizing using Scofield relative sensitivity factors.¹²⁶

Figure 10. Example of the first seven abstract factor images used to reconstruct the image-data set for the imaging XPS part in **Paper II**. The data-image set is separated into abstract factor images that contains the largest eigenvalues and are used to enhance the images with a higher signal to noise ratio.

2.4 Near edge X-ray absorption fine structures spectroscopy (NEXAFS)

NEXAFS is a surface sensitive technique that can obtain information of the electronic structure and orientation of molecules. This is achieved by scanning the photon energy over the absorption edge of the element present and probing the excitation of core level electrons into unoccupied molecular orbitals or continuum states. NEXAFS shows strong and distinctive features in the energy region just below and after the absorption edge.¹²⁷ During NEXAFS measurement, electrons can be detected in total electron yield (TEY) and partial electron yield (PEY). In TEY, all the electrons that emerge from the specimen are measured through the current from the sample. For PEY, which is a more surface sensitive mode, a retarding electric field is applied in front of the detector, which suppresses low energetic electrons and only detecting electrons from the top outermost layer.¹²⁸ NEXAFS measurements have been used in this thesis to investigate the change in oxidation states for the cerium oxide nanoparticles in **Paper III** and on Gd_2O_3 and Eu_2O_3 nanoparticles spin-coated on Si substrate in **Paper V**.

2.5 Mirror electron microscopy (MEM)

In MEM, an electron beam is used as an irradiation source where an electron mirror is used as a conducting sample surface that being biased with a negative potential similar or slightly more negative than the electron beam. This generates de-accelerated electrons that do not reach the sample surface before being turned away. This technique is unique in that way that the sample is not bombarded by electrons or emits electrons. From MEM measurements, information of the sample topography is being obtained by these reflected electrons where the contrast arises from local micro fields at the surface.¹²⁹

2.6 Low energy electron microscopy (LEEM)

In LEEM, an applied potential at the surface is slightly less negative than the electron beam, making only electrons with low energy interact with the sample surface as compared to MEM, where the applied potential is more negative than the electron beam. Elastically backscattered electrons from the sample surface are used for imaging. Low energy electron diffraction (LEED) patterns can also be obtained using this method. The lateral resolution for LEEM is high i.e. less than 10 nm which makes it ideal for imaging flat homogenous surfaces.^{129, 130}

2.7 Fluorescence microscopy

Fluorescence microscopy is an optical technique and widely used in biology for providing images of labeled target structures. Fluorescent labeling is needed to provide any information. There exist several labeling techniques e.g., stains and probes¹³¹, immunofluorescence¹³², and fluorescent protein expression¹³³, to name a few. The principle is based on fluorescence, a phenomenon where a material/fluorophore can absorb light and then emit light with a longer wavelength. When a fluorophore absorbs light, it is excited from the ground state (S_0) to a higher excited state (S_1). At the higher energy state, the electrons undergo vibrational relaxation and internal conversions and return to the ground state. Through this return to the ground state, the fluorophore emits light with a longer wavelength, i.e. fluorescence. The shift in wavelength between the excited and emitted light is called Stokes shift^{134, 135} and is illustrated in Figure 11.

Typical components in a fluorescence microscopy setup are a light source, a set of optical filters i.e. excitation- and emission filter and a dichroic beam splitter and objectives. The working mechanism of a typical fluorescence microscope is shown in Figure 12. The excitation filter selects the wavelength of the light used to excite the fluorophore in the sample. The filtered excitation light is then reflected towards the sample by a dichroic beam splitter. The dichroic beam splitter also transmits the emitted light from the sample through to the detector. An emission filter is placed at the end to ensure that any residual excitation light is filtered out.¹³⁴ During this thesis work, we have used fluorescence microscopy as a complementary technique to PEEM and imaging XPS, since it is a well-established and frequently used in biology.

Figure 11. Jablonski diagram illustrating the principle of fluorescence with the transition from ground state to higher electronic states and by internal conversion and vibrational relaxing back to the ground state with the result of fluorescence. The Stokes shift is illustrated between the excitation and the emission wavelength.

Figure 12. Schematic illustration of the working mechanism of fluorescence microscopy.

2.8 Transmission electron microscopy (TEM)

Transmission electron microscopy is a microscopy technique with a set up similar to an optical microscope where the main differences are in the lenses and light source. For TEM, magnetic lenses and electrons are used as illumination source compared to glass lenses and light sources for an optical microscope. In TEM, a beam of electrons is sent towards a very thin sample (<100 nm) where electrons are transmitted through the sample or inelastically or elastically scattered.¹³⁶ Because of the high energy of the electrons (100-400 keV) and consequently short wavelength of the electron beam, TEM can image samples with sub-nanometer resolution. The use of TEM in biology have been an important technique for analysis and visualization of cellular components.¹³⁷⁻¹⁴⁰ In this thesis, TEM has been used as benchmarking technique for comparison with NanoESCA imaging on ultrathin slices of neutrophils.

3. Key results

3.1 Neutrophil imaging using PEEM and imaging XPS

Images of neutrophils attached on silicon oxide substrates have been acquired using the NanoESCA to show the potential of a combined PEEM and imaging XPS instrument as an analytical tool for visualization and characterization of biological materials. Energy filtering allows for acquiring images over the photoemission threshold, revealing contrast based on work function variations on the sample and from the sample topography. In Figure 13, (**Paper IV**) threshold images have been obtained by scanning the photokinetic energy from $E-E_F = 5.5-0$ eV using an Hg arc lamp, where the entire and also part of the scan is summed into single images i.e. $E-E_F = 5.5-0$ eV, $E-E_F = 5.5-3.9$ eV and $E-E_F = 5.5-0$ eV. The use of energy-filtered PEEM makes it possible to not only acquired energy-filtered images but also enables easy sample navigation with FoV from $620\text{ }\mu\text{m}$ down to $3\text{ }\mu\text{m}$.

Inactivated neutrophils circulating in the bloodstream with a spherical shape, but upon activation, they can change their shape to a more flat morphology. This is a first step before transferring from the blood and into the extracellular tissue space.⁷⁵ From the energy-filtered PEEM images, the neutrophils show a flat shape on the silicon oxide substrate with an enlarged surface and extended pseudopods. Thin tubulovesicular extensions are also visible in the images. The characteristic polymorphonuclear morphology i.e. with the distinctive lobulated nucleus, is clearly visible in the center of the cells and can be highlighted by only presenting a part of the photokinetic energy scan, i.e. $E-E_F = 3.9-0$ eV.

We have compared our energy-filtered PEEM images with a benchmarking technique in biology, namely fluorescence microscopy. The neutrophils have been stained with SYTOX Green, a commonly used fluorophore for dead cell staining of nucleic acids.¹⁴¹ The comparison between energy-filtered PEEM and fluorescence microscopy images of the core reveals a clear contrast of the polymorphonuclear morphology.

Figure 13. Neutrophils attached on a silicon surface and imaged at three areas (a-c) using energy-filtered PEEM. The obtained images are the sum of low energetic secondary electrons that have been acquired over the photoemission threshold from I) $E-E_F = 5.0-0$ eV, II) $E-E_F = 5.0-3.9$ eV, and III) $E-E_F = 3.9-0$ eV. IV) Fluorescence microscopy images of SYTOX green stained DNA.

The possibility to both image low energetic secondary electrons and core level electrons with contrast from local work function and spatially resolved element specific information in the form of elemental maps brings forward two different dimensions which delivers a more comprehensive view of the actual sample. This have been achieved and shown for two neutrophils attached on a silicon oxide substrate by acquiring energy-filtered PEEM images of low energetic secondary electrons using the Hg arc lamp, and imaging XPS images of core level electrons using a monochromatized Al $K\alpha$ X-ray source. These are shown in Figure 14 (**Paper IV**). XPS C1s, Si2p, N1s, O1s and P2p core level electrons have been acquired and processed using PCA based algorithms to increase the signal to noise ratio. The imaging XPS results are presented as atomic percent distributed core level maps. The images have been obtained via a quantification on a pixel by pixel basis by measuring the peak areas with a linear background and normalized using Scofield relative sensitivity factors. The spatial distribution from the XPS core level electrons show good contrast for the two neutrophils. There is a clear C1s signal for the two neutrophils with features comparable with the energy-filtered PEEM images. The C1s signal is also distributed over the entire field of

view (FoV) probably due to contaminations from exposure to the incubation solution of the cells. The Si2p signal is observed as the inversion of the C1s signal. XPS N1s and P2p clearly show presence of nitrogen and phosphor and well resolved for the two neutrophils. The XPS O1s signal is distributed over the entire FoV, although with a higher signal from the silicon oxide substrate.

The ability to perform spectromicroscopy and extract local spectra for a well-defined region is a strength when using the NanoESCA. In addition to elemental maps, XPS core level spectra have been extracted for one of the neutrophils and for the substrate signal of the silicon oxide substrate. Extracted XPS spectra for the neutrophil sample area showed presence of oxygen, phosphor, nitrogen, and carbon. Comparing with the substrate, where XPS results showed presence of silicon, oxygen, and carbon.

Figure 14. Sum of energy filtered PEEM images of low energetic secondary electrons over the photoemission threshold of two attached neutrophils (a) and extracted XPS core level spectra b) O1s, c) P2p, d) C1s, e) Si2p and f) N1s of one of the neutrophil (black) and the substrate (red) with corresponding elemental maps of the atomic distribution of (g) C1s, (h) Si2p, (i) N1s, (j) O1s and (k) P2p.

A comparison between attached neutrophils on gold and silicon using energy-filtered PEEM have been done (**Paper IV**) and is shown in Figure 15. The work function for gold and silicon at the interface has been mapped out and we have measured a shift in work function of 0.3 eV between gold and silicon. Clean gold and clean silicon oxide have a higher work function value ¹⁴², but due to incubation of attached neutrophils and extensive but careful rinsing, possible contaminations will lower the work function values for both gold and silicon. The photoemission threshold for the neutrophils have a maximum about 4.4 eV which is in between the work function distribution of gold and silicon, and this is shown in the energy-filtered PEEM images with a darker contrast for the neutrophils at gold areas and brighter for areas where the neutrophils are attached on silicon oxide. In both examples, the characteristic polymorphonuclear morphology is clearly visualized in the center of the neutrophils.

Figure 15. Energy-filtered PEEM images acquired at a) $E-E_F = 4.6 \text{ eV}$ and b) $E-E_F = 4.3 \text{ eV}$ for the interface between gold and silicon with corresponding work function map (c). Attached neutrophils on gold (d-f) and silicon (g-i) obtained by the sum of low energetic secondary electrons from $E-E_F = 5.5\text{-}2.0 \text{ eV}$.

Ultra-thin sections ($\sim 60 \text{ nm}$) of neutrophils have been prepared and deposited onto silicon oxide surfaces to image and map the interior of the neutrophils using energy-filtered PEEM, shown in Figure 16 (**Paper IV**). TEM images of neutrophils have been deposited onto formvar coated slot grids and acquired to be used as a comparison, since TEM is frequently being used for imaging of biological materials. The comparison of the obtained images

between energy-filtered PEEM and TEM, where both techniques obtained distinct contrast between the cell nucleus with segregated lobules. Since these are ultra-thin sections, the inside of the lobules is visualized with a contrast for the more highly condensed heterochromatin shown darker than the brighter more lightly packed euchromatin.⁷¹ Work function maps have been created from the local work function values acquired over the photoemission threshold from $E-E_F = 5.5-3.0$ eV.

Figure 16. Ultra-thin slices of neutrophils characterized using TEM (a-c) and energy filtered PEEM (d-g) with corresponding work function maps (h-k).

Neutrophils and the interaction with controlled exposure of QDots and subsequent release of NETs have been imaged using PEEM and imaging XPS (**Paper II**). Neutrophils have been attached on a gold patterned silicon substrate and imaged in the presence of QDots, shown in Figure 17. NETs are clearly visualized using PEEM and we have shown by using energy-filtering PEEM that local contrast within the NETs is achieved at lower kinetic energy of the photoemitted electrons. This contrast is due to assembly of QDots, with a change in the chemical environment and elemental composition and this have been further shown in good agreement with images obtained by fluorescence microscopy (Figure 1, **Paper II**). The distribution of elements has been acquired using imaging XPS. XPS core level spectra and elemental maps have been acquired over the entire FoV to investigate the elemental distribution. Cadmium (Cd) has been used for tracking of the QDots by scanning over the XPS $Cd3d_{5/2}$ peak. The presence of Cadmium is located at the position of the NETs structures where the signal intensity is distributed along the network. The distribution of C1s is located for all the neutrophils and at the NETs. Elemental contrast from both $Cd3d_{5/2}$ and C1s are thus distinguishable at the position of the NETs with clear contrast of the network structure. Overlaid color-coded core level images provides a mean to produce maps of the elemental distribution to highlight the presence of each element where O1s (blue), $Cd3d_{5/2}$ (red) and C1s (green) have been overlaid in one image and Au4f_{7/2} (yellow) and Si2p (grey) in another image to highlight the gold pattern silicon substrate.

Figure 17. Energy-filtered PEEM and imaging XPS of NETs in the presence of QDots have been obtained using an AlK α X-ray source ($h\nu = 1486.6$ eV) and acquired at a) $E-E_F = 3.9$ eV, b) $E-E_F = 3.0$ eV. Elemental maps showing the distribution of core level electrons have been overlaid and color coded in c) O1s, C1s and Cd3d_{5/2} and d) Si2p and Au4f. Adapted with permission from Skallberg et al.⁵ Copyright 2020 American Chemical Society.

3.2 QDot linking to functionalized gold patterned silicon substrate

Specific attachment of QDots to self-assembled monolayer on a gold pattern silicon substrate have been used as a template to show PEEM and imaging XPS capabilities for patterned bio-relevant samples (**Paper I**). We have performed stepwise functionalization of a self-assembled monolayer from a solution of alkane thiols (i.e. 16-mercaptohexadecanoic acid, MHDA) onto the gold patterns. Adsorption of alkane thiols is a well-established technique to form self-assembled monolayers on gold.¹⁴³ MHDA self-assembled onto gold pattern was followed by EDC/NHS (1-Ethyl-3-(3-dimethylaminopropylcarbodiimide)/N-hydroxysuccinimide) activated PEG (polyethyleneglycol)-diamine. The EDC/NHS carbodiimide coupling strategy is common method for covalently bound carboxylic acids with primary amines.¹⁴⁴

As a last step, The MHDA-PEG-diamine functionalized gold pattern silicon substrate was further functionalized with Eu-YVA QDots using EDC/NHS. The MHDA-PEG-diamine-Eu-YVA QDots sample have been analyzed with energy-filtered PEEM and Imaging XPS and is shown in Figure 18.

An energy-filtered PEEM image acquired at $E-E_F = 4.4$ eV showing a part of the sample surface with clearly visualized gold patterns. The contrast in the image is due to a difference in the work function between gold and silicon. Elemental imaging of core level electrons has been performed in a smaller FoV, for two of the gold patterns and is highlighted with a box in Figure 18a. The element distribution of silicon, gold, carbon, oxygen and vanadium have been obtained by scanning the photoelectron kinetic energy across XPS Si2p, Au4f_{7/2}, C1s, V2p and O1s and quantified by a pixel-by-pixel by analysis by measuring the peak areas using a linear background and normalized with Scofield sensitivity factors.¹²⁶

The successful self-assembled monolayer of thiols to gold with specific attachment of Er-YVA QDots is demonstrated by the elemental maps. XPS C1s and V2p are spatially resolved and detected on the gold squares. Strong XPS Si2p, Au4f_{7/2} and O1s signals are obtained from the gold pattern silicon substrate.

Figure 18. a) An energy-filtered PEEM image of the gold pattern silicon substrate acquired at $E-E_F = 4.4$ eV using a Hg-lamp ($h\nu = 5.2$ eV). Imaging XPS for the marked area in (a) showing the elemental distribution of b) Si2p (grey) and Au4f_{7/2} (yellow) and c) C1s (green), O1s (blue) and V2p (red). Copyright 2020 American Vacuum Society.

3.3. Cerium oxide nanoparticles with gadolinium integration

Cerium nanoparticles with various amount of gadolinium integrated have been synthesized to be used as a contrast agent with antioxidant properties for MRI (**Paper III**). Seven samples have been prepared with increasing amount of gadolinium namely, CeOx, CeOx:Gd9%, CeOx:Gd19%, CeOx:Gd32%, CeOx:Gd41% and CeOx:Gd46%.

The antioxidant properties of cerium are due to the fact that cerium can switch between Ce^{4+} and Ce^{3+} oxidation states and this is correlated to the key processes for the scavenging properties of the nanoparticles.⁴⁷ Depending on the environment, e.g. in presence of ROS, the oxidation state can be switched. The change in oxidation states have been characterized at the synchrotron storage ring MAXII, at beamline D1011, MAX-LAB in Lund, by using NEXAFS measurements. NEXAFS, as described in section 5, can probe the excitation of core level electrons into unoccupied molecular orbitals or continuum states. This is useful for e.g. quantification of oxidation states. The changes in oxidation states for cerium have been obtained by scanning the absorption of Ce M-edge of three synthesized nanoparticle samples, 1) CeOx, 2) CeOx:Gd46% and 3) CeOx:Gd46% with hydrogen peroxide (H_2O_2) as a ROS to demonstrate the antioxidant properties of the nanoparticles. For spectral component analysis, two reference samples, CeO_2 nanopowder (Ce^{4+} oxidation state) and Ce(III) acetate (Ce^{3+} oxidation state) have also been measured to be used as single components for each oxidation state and are shown in Figure 19. The 3+ oxidation state is increasing for CeOx:Gd46% when implementing more Gd as compare to CeO_2 with no Gd. When introducing H_2O_2 to CeOx:Gd46% the oxidation state shifts towards a major 4+ component with a reduced 3+ component. This is a strong indication that the nanoparticles can function as an active antioxidant with ROS scavenging properties.

Figure 19. NEXAFS spectra obtained by scanning the absorption of the Ce M-edge for a) CeO_2 , b) Ce(III)acetate , c) CeOx nanoparticles, d) $\text{CeOx:Gd46\%}$ and e) $\text{CeOx:Gd46\%}$ with H_2O_2 . This figure is adapted from the original figure, which was published by Eriksson et al.¹⁴⁵ and is licensed under the Creative Commons attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).

3.4 Eu-doped Gd₂O₃ nanoparticles catalyzed SiO_x annealing

The annealing temperature for native oxide removal of silicon and simultaneously formation of silicide has been studied (Paper V). A low coverage of Eu-doped Gd₂O₃ nanoparticles was deposited on a clean silicon substrate and stepwise annealed from 500 up to 830°C. We have used MEM, LEEM, XPEEM and NEXAFS synchrotron based surface sensitive techniques at the storage ring MAX II, beamlines D1011 and I311, MAX-LAB in Lund for characterization of annealed silicon substrate with and without Eu-doped Gd₂O₃ nanoparticles.

MEM characterization of the sample topography for native oxide removal of a clean silicon substrate without any deposited nanoparticles shows void formation at a temperature of 800°C, whereas with nanoparticles, the void formation starts already at 500°C, shown in Figure 20. The annealing process is triggered by the presence of Eu-doped Gd₂O₃ nanoparticles resulting in an activated oxide removal at lower temperatures. XPEEM measurements at 830°C of Si2p, Gd4f and Eu4f shows a separation of Eu and Gd on the silicon substrate. The nanoparticles show a fragmentation during annealing with a temperature dependent migration of Eu and Gd, where Eu is located at areas with clean silicon and Gd located at areas with SiO_x. At lower temperatures, about 500°C, it has been shown that reactions between rare-earth elements and silicon are commonly observed by creating rare-earth silicide.¹⁴⁶ A comparison between local extracted photoemission Si2p spectra from Gd rich SiO_x areas (blue) and Eu rich Si areas (red) reveals a shift towards lower binding energies for the Eu rich Si areas and is attributed to formation of superficial Eu-Si. This is in good agreement with other studies of rare-earth Si reactions.¹⁴⁷ The removal of native oxide at lower annealing temperatures and subsequent formation of europium silicide is a promising one step energy saving strategy of producing silicon-based contacts.

Figure 20. MEM images of a) silicon substrate with deposited Eu doped Gd₂O₃ nanoparticles, annealed at 500°C, showing initial void formation. Silicon substrate with no nanoparticles annealed at b) 700°C and c) 800°C, with void formation starting at 800°C. This figure is adapted from the original figure, which was published by Selegård et al.¹⁴⁸ and is licensed under the Creative Commons attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).

Figure 21. Characterization of Eu doped Gd_2O_3 nanoparticles deposited on a silicon surface heated at 830°C . a) MEM image with inset LEED pattern, b) XPEEM of SiO_x (BE 102.5 eV), c) XPEEM of $\text{Si}2p$ (BE 99.5 eV), XPEEM of $\text{Si}2p$ (BE 102.5 eV), XPEEM of $\text{Gd}4f$ (on resonance), and e) XPEEM of $\text{Eu}4f$ (on resonance). This figure is adapted from the original figure, which was published by Selegård et al.¹⁴⁸ and is licensed under the Creative Commons attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).

Figure 22. a) Local extracted photoemission $\text{Si}2p$ spectra from SiO_x rich areas (blue) and clean Si (red) with corresponding XPEEM image of $\text{Si}2p$. This figure was published by Selegård et al.¹⁴⁸ and is licensed under the Creative Commons attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).

3.5 Ultra-small Fe₃O₄ nanoparticles as contrast agent for MRI

Ultra-small Fe₃O₄ nanoparticles have been synthesized to be used as both a positive (T₁) and a negative (T₂) contrast enhancement agent for MRI (**Paper VI**). Synthesized Fe₃O₄ nanoparticles that utilizes both of T₁ and T₂ simultaneously to enhance the local contrast will improve the sensitivity in the diagnostics of diseases. The ultra-small Fe₃O₄ nanoparticles (1.7, 2.2 and 4.5 nm) have been prepared by a one-step coprecipitation method in the presence of poly(acrylic acid) (PAA), where PAA works as a stabilizer of the nanoparticles and as a crystal growth inhibitor. The carboxylic acid groups of PAA coordinates to Fe³⁺ ions at the surface during formation of Fe₃O₄ nanoparticles to form a hydrophilic layer.

XPS have been used as a complementary technique to characterize the composition of the Fe₃O₄ nanoparticles and the coordination of PAA, shown in Figure 23. To further identify in which crystalline form the as-synthesized nanoparticles consist of XPS have been used since Fe₃O₄ and γ -Fe₂O₃ in e.g. X-ray diffraction pattern are similar. XPS is sensitive to and can differentiate Fe³⁺ from Fe²⁺. XPS measurements were performed to investigate the chemical composition of as-synthesized Fe₃O₄ nanoparticles (2.2 nm). XPS wide scan confirmed the presence of iron, oxygen, and carbon. High resolution XPS Fe2p have been acquired with binding energy peak positions for Fe2p_{3/2} and Fe2p_{1/2} located at about 710.8 and 724.6 eV. This is in good agreement with previous studies on Fe₃O₄ nanoparticles.¹⁴⁹ For the γ -Fe₂O₃ form characteristic binding energies peak positions for Fe2p_{1/2} and Fe2p_{3/2} are expected at about 710.4 and 724.7 eV, respectively.¹⁵⁰ This indicates that the product is in the form of Fe₃O₄ rather than γ -Fe₂O₃. The XPS The C1s spectrum have been deconvoluted into three peaks at binding energy position 285.0, 287.0 and 288.9 eV and we have assigned them to -C-C-, -C-OH and -C-OOH groups. These results are in good agreement with previous studies on coordination of carboxylic acid groups to Fe₃O₄ nanoparticles.¹⁴⁹

Figure 23. a) XPS wide scan, and b) high resolution XPS Fe2p and c) C1s of 2.2 nm sized Fe₃O₄ nanoparticles. Wang et al.¹⁵¹, Adapted by permission of The Royal Society of Chemistry.

4. Summary of papers

Paper I

Andreas Skallberg, Caroline Brommesson and Kajsa Uvdal, *Imaging XPS and photoemission electron microscopy; surface chemical mapping and blood cell visualization*, **Biointerphases Vol. 12, Issue 2, 2017**

This paper examines the use of a combined photoemission electron microscopy (PEEM) and imaging X-ray photoelectron spectroscopy (XPS) for surface characterization of bio-relevant and biological samples. Element specific core level images, chemical shifts, threshold mapping with work function contrast and topographical information. Three samples have been prepared and characterized to show the potential of combined PEEM and XPS imaging. 1) Stepwise surface modification by self-assembled monolayers to a gold pattern silicon substrate, followed by specific linking of europium doped yttrium vanadate colloid (Eu-YVA) Quantum dots. 2) Neutrophils on silicon surface and 3) platelet adhesion to immunoglobulin G modified silicon surface.

Paper II

Andreas Skallberg, Kalle Bunnfors, Caroline Brommesson and Kajsa Uvdal, *Neutrophils Activated by Nanoparticles and Formation of Neutrophil Extracellular Traps: Work Function Mapping and Element Specific Imaging*, **Analytical Chemistry, Vol. 91, Issue 21, 2019**

In this paper, we have visualized neutrophil interaction with cadmium selenide (CdSe) zinc sulfide (ZnS) core-shell quantum dots (QDots) and the release of neutrophil extracellular traps (NETs). Imaging XPS and PEEM have been used to obtain images of neutrophils before and just after release of NETs with local work function contrast and element specific images. We show elemental composition with laterally resolved XPS core level images and work function variations for localization of QDots at the position of the neutrophils and the released NETs. This work shows the potential of PEEM and imaging XPS as advanced analytical tools for future research on cellular activities and nanoparticle tracking.

Paper III

Peter Eriksson, Alexey Tal, **Andreas Skallberg**, Caroline Brommesson, Zhang-Jun Hu, Robert Boyd, Weine Olovsson, Neal Fairley, Igor Abrikosov, Xuanjun Zhang and Kajsa Uvdal, *Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement*, **Scientific Reports, Vol. 8, Issue 1, 2018**

In this study, we have prepared and examined cerium oxide nanoparticles with integrated gadolinium to be used as an MRI contrast enhancer with antioxidant properties. Cerium oxide nanoparticles were synthesized with 0-46% gadolinium integration and characterized by means of the crystal structure, size, shape, colloidal stability and relaxivity. The change in oxidation states for cerium oxide were characterized at MAX LAB using near edge X-ray absorption fine structures spectroscopy (NEXAFS) in combination with density functional

theory (DFT) calculations. Reference spectra from Ce^{3+} and Ce^{4+} were used for spectral peak fitting of the synthesized nanoparticles. *In vitro* models of human neutrophils, PMA and dichloro-dihydro-fluorescein (DCF-DA), were used for the determination of the antioxidant properties.

Paper IV

Andreas Skallberg, Kalle Bunnfors, Caroline Brommesson and Kajsa Uvdal
New Tools for Imaging Neutrophils: Work Function Mapping and Element Specific, Label Free Imaging of Cellular Structures, **Submitted to Nano Letters**

In this work, we have visualized neutrophils and their cellular morphology on both gold and silicon substrates using PEEM and imaging XPS. Sum of low energetic secondary electrons images from threshold scans using energy-filtered PEEM clearly shows the characteristic polymorphonuclear morphology of the nucleus, where the nuclei is segregated into 2-5 lobules. We have demonstrated work function variations in imaging mode for the cellular core of the neutrophils and element specific XPS core level images. Fluorescence microscopy and ultra-thin slices for TEM have been used as benchmarking techniques for comparison to pinpoint the potential of using PEEM and imaging XPS for cellular imaging.

Paper V

Linnéa Selegård, Andreas Skallberg, Alexei Zakharov, Natalia Abrikosova and Kajsa Uvdal
Step by step Rare-Earth catalyzed SiO_x annealing and simultaneous formation of Europium-silicide by low coverage of Eu doped Gd_2O_3 nanoparticles. **Surface Science, 2020**

In this study, we have investigated the oxide removal of a SiO_x surface and formation of silicide using a low coverage of europium doped gadolinium oxide nanoparticles during annealing. We report a significant reduction in the annealing temperature for oxide removal with presence of Eu doped Gd_2O_3 nanoparticles. During the annealing process, fragmentation of the nanoparticles occurs where Gd migrates to SiO_x rich areas, whereas Eu migrates to clean Si to form Eu based silicide. This shows a promising one step and energy saving way of producing silicon based contacts.

Paper VI

Guannan Wang, Xuanjun Zhang, Andreas Skallberg, Yaxu Liu, Zhangjun Hu, Xifan Mei and Kajsa Uvdal, *One-step synthesis of water-dispersible ultra-small Fe_3O_4 nanoparticles as contrast agents for T_1 and T_2 magnetic resonance imaging*, **Nanoscale, Volume 6, Issue 5, 2014**

Here we report synthesized, water-dispersible, and uniform ultra-small Fe_3O_4 nanoparticles as contrast enhancers in MRI. The synthesized nanoparticles showed good colloidal stability in physiological solutions and good biocompatibility in cell viability assays. The nanoparticles showed high relaxivity compared to commercially available contrast agents and as a potential T_1 and T_2 dual MRI contrast agent. *In vivo* and *in vitro* imaging, the ultra-small Fe_3O_4 nanoparticles exhibited long circulation times, good contrast enhancement and low toxicity.

5. Conclusion and future perspective

The research presented in this PhD thesis is focused on photoemission-based surface sensitive techniques for bioimaging, including characterization of cellular structures using (with and without) nanoprobe. Advanced instrumental tools such as PEEM and imaging XPS are commonly used for laboratory-based measurements for analysis/material characterization within surface physics and material sciences. The aim herein is to investigate its potential as a powerful technique for interdisciplinary research. These techniques have in this thesis been used to demonstrate its potential for imaging biological processes on the cellular level. In this work, we investigate the possibilities to bridge between material sciences and life science. Characterization of neutrophils and their response when activated by nanomaterial/particles have been performed. The main instrument used in this work is the so called NanoESCA where the techniques of PEEM and imaging XPS are combined, each with their own unique capability to provide spatially resolved information. In PEEM, images are generated in the nanoscale dimension with work function contrast based on local variations in the electron emission. Core level electrons are acquired using Imaging XPS to obtain maps of the elemental distribution and chemical shifts at sub micrometer dimension. We have demonstrated element specific label free imaging of cellular structures, work function variations of the polymorphonuclear morphology and extracellular structures. These results demonstrate that the NanoESCA instrument is promising for cellular studies, where element specific information is required.

To deliver comprehensive picture of biological samples, a combination of analytical techniques would be desirable to use for elemental and chemical mapping. Further investigation using a set of complementary and correlative techniques to PEEM/imaging XPS. Also, the use of synchrotron-based instruments such as XPEEM and NEXAFS microscopy for chemical imaging, at national infrastructures such as MAXIV may contribute with detailed information by probing the surface chemistry with high chemical sensitivity. We have further shown that the use of electron microscopy (TEM) in combination with PEEM may contribute with valuable information such as detailed morphological structures and changes due to cell activation/inflammation of the cell. The ability to image inside a cell will be further investigated, with high resolution elemental and chemical maps which may enable further improved visualization strategy, higher resolution with increased sensitivity of local compositions of complex structures.

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