Leibniz IPHT
LEIBNIZ INSTITUTE of PHOTONIC TECHNOLOGY

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FINAL PROJECT WORKSHOP

„TRACKING AND ASSESSING THE RISK FROM ANTIBIOTIC RESISTANT GENES USING CHIP TECHNOLOGY IN SURFACE WATER ECOSYSTEMS (TRACE)“

DECEMBER 7, 2017 // 09:00

Leibniz IPHT Jena (Beutenberg Campus) // Foyer // Albert-Einstein-Str. 9 // 07745 Jena

PROGRAM

09:00 Welcome & Introduction // Wolfgang Fritzsche

ANTIBIOTIC RESISTANCE // Wolfgang Fritzsche

09:10 The burden of multi-resistant Gram-negative bacteria (MRGN)
Oliwia Makarewicz // Jena University Hospital, Germany

09:35 Tetracycline Resistance – General Properties and Specific Features in Chlamydia
Christian Berens// Federal Reseach Institute for Animal Health, Jena

10:00 Detection of Waterborne Pathogens in Hospital-Related Water Using Next-Generation Sequencing Technologies
Silvia Garcia Cobos// University Medical Center Groningen, Netherlands

10:25-11:00 COFFEE BREAK

CONTAMINANTS IN RAW WATERS // Enda Cummins

11:00 Indicators or Pathogens – Which Parameters are Suitable for a Microbiological Risk Assessment of the Raw Water?
Hartmut Willmitzer // Thuringian Reservoir Adm., Germany

11:25 Quantification of Antibiotic Resistant E. coli in two River Systems of Central Italy
Angelo Solimini // Sapienza University, Rome, Italy

11:50 Impact of Wastewater Discharges on the Resistome and the Mobilome of Surface Waters (the Saale River and the Ter River as Model Systems)
Carles Borrego // Catalan Water Institute Girona, Spain

12:15-13:00 LUNCH BREAK

HUMAN EXPOSURE // Angelo Solimini

13:00 Human Exposure to Antibiotic Resistant Escherichia coli through Drinking and Irrigation Water Sources.
Eithne O'Flaherty // UCD, Ireland

13:25 Analysis of Water, Food and Antibiotics in a Commercial Lab
Bernd Giese // Synlab, Jena, Germany

13:50 Antibiotics and Resistance in Waste Water
Peter Krebs // TU Dresden, Germany

14:15-15:00 COFFEE BREAK

MOLECULAR DETECTION APPROACHES // Wolfgang Fritzsche

15:00 A Simple Spectrophotometer for Colorimetric DNA Assays
Matthias Urban // Leibniz IPHT Jena, Germany

15:25 Paralleled Identification of DNA by Localized Surface Plasmon Resonance (LSPR) by an Imaging Spectrometer
David Zopf // Leibniz IPHT Jena, Germany

16:00 End

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The burden of MRGN

Oliwia Makarewicz
Jena University Hospital, Germany

β-Lactamases are ancient and independently developed enzymes in many species that hydrolyse β-lactam antibiotics, which are also natural products of some microorganisms. Thus, β-lactamases are a highly inhomogeneous group of enzymes with currently more than 1,500 described variants that can be classified either according to their protein sequence homology (Ambler classification) or their phenotypic profile (Bush-Jacoby-Medeiros classification).
The spread of multi-resistant Gram-negative bacteria (MRGN) producing extended-spectrum β-lactamases (ESBLs) became a rising problem worldwide for the last two decades. The main reasons for this trend are a cross-sectorial overuse of the β-lactam antibiotics that fosters the spread of MRGNs, but also the association of the β–lactamase genes with mobile elements such as integrons and plasmids, which accelerate interspecies transfers of the resistance genes and their mutagenesis and adaptation to selective environments. These induced a unique diversification of the β–lactamases compared to other resistance determinates, which is a high burden for routine diagnostics, antimicrobial therapy and epidemiological tracking. Besides increasing rates of ESBL-infection, increasing rates of gut colonisation by ESBL-producing MRGN are observed in humans and animals that have been shown to increase the risk of ESBL-infections. ESBLs contaminations have been also identified in surface waters, in the soil and on vegetables raising a new concern of a ‘resistance pollution’ of the environment that seems to be jointly responsible for the observed increasing prevalence of MRGN. Thus, there is a need for improved monitoring and controlling of the MRGN not only in the health-care systems, but also in other sectors, such as livestock farming and agriculture, sewage systems and groundwater, etc. These aspects will be discussed in this lecture.

Tetracycline resistance – general properties and specific features in *Chlamydia*

Christian Berens
Friedrich-Loeffler-Institute - Federal Reseach Institute for Animal Health, Jena

Tetracyclines have seen widespread usage in human and veterinary medicine as well as in agriculture – to the extent that they have been considered environmental contaminants and that antimicrobial resistance has increased dramatically with multiple determinants having been found in resistant bacterial isolates. Drug efflux is a major resistance mechanism found in many different bacterial species and is frequently associated with mobile elements on multi-resistant plasmids. Efflux-based resistance determinants, termed Tet(xyz), are tightly regulated at the transcriptional level so they do not impose a fitness disadvantage on their host. One determinant, Tet(C), has been identified in *Chlamydia suis*, an obligate intracellular pathogen frequently found in pigs. This Tet(C) variant has several unusual properties, such as only a low-level resistance to tetracycline, an apparently constitutive activity and a so far unknown origin. Unraveling the mechanistic basis of these peculiarities will help us better understand both tetracycline resistance and chlamydial physiology.

Detection of waterborne pathogens in hospital-related water using next-generation sequencing technologies

Silvia García-Cobos, Giuseppe Fleres, Natacha Couto, Mariëtte Lokate, John W. Rossen, Alex W. Friedrich
University of Groningen, University Medical Center Groningen, The Netherlands.

Background: Humid environmental compartments connected to the hospital water system can serve as a reservoir of health-care associated pathogens and are involved in their transmission, being one of the major problems in infection prevention and control. One of the most common water-borne pathogens is *Legionella spp*, which major route of transmission is through inhalation or aspiration of contaminated aerosols. These aerosols are highly produced in a dental unit environment what could pose a risk for immunocompromised patients. This study aimed to assess the presence of *Legionella* spp. and other potential pathogens in water from dental chairs of a hospital dental unit using different next-generation sequencing techniques.
Materials/methods: *Legionella anisa* contamination was observed by culturing ($1x10^2$ CFU/mL) in three hospital dental chairs. wgMLST typing results indicated that all strains (n=4, two isolates from the same chair) belonged to the same clon. In addition, water samples (tap and water-syringe) (n=8) collected from four dental chairs, including the contaminated ones were analysed using amplicon-based sequencing (AmplBS) and shot-gun metagenomics (SGM). The samples were filtered (0.2mm pore size) and the DNA was extracted (PowerWater kit, MoBio). Libraries were prepared directly from the DNA (SGM) or after PCR amplification (16S and 16S-23S rDNA regions) using the Nextera XT kit (Illumina). Libraries were sequenced on a MiSeq instrument (Illumina; 500-cycles [SGM] or 600-cycles [AmplBS], paired-end). CLC Genomics Workbench v10.1.1 (Qiagen) was used for quality trimming, OTU clustering of AmplBS data (in-house database for 16S-23S region; 23,377 sequences) and for *de novo* assembly of SGM data. Microbial composition and antibiotic resistance genes (ARGs) (> 70% identity; 80% coverage) of SGM data were also determined using One Codex and ABRicate v0.7, respectively. A cut-off value of 1% relative abundance (RA%) was applied.
Results: Regarding opportunistic pathogens, from SGM data we identified nontuberculous (NT) *Mycobacterium* spp. (n=8, 1.2-27 RA%) (*M. gordonae* being the most abundant; n=4, 1.4-2.4%), *Burkholderia* spp. (n=5, 1.1-8.3 RA%), *Pseudomonas* spp. (n=4, 1.1-1.3 RA%) and *Leptospira* spp. (n=3, 3.8-48 RA%). The analysis of 16S-23S rDNA data revealed only NT Mycobacterium spp. (n=1, 1.21 RA%). None of the above microorganisms were detected by 16S rDNA sequencing (table). *Legionella* spp. showed values below the cut-off 1% in all samples. Regarding ARGs, *mur*A (n=1, intrinsic fosfomycin resistance) and *mds*B (n=1) and *gol*S (n=1) (related to multidrug efflux-pumps) were found in the metagenomes.
Conclusion: The identification of a unique clone of *L. anisa* in several chairs in the same hospital dental unit may indicate a common contamination source in the dental unit waterlines over a prolonged period of time. Amplicon-based sequencing analysis showed low sensitivity for detecting waterborne pathogens. Pathogen detection, at both genus and species level, together with information on ARGs, virulence factors and plasmid content by SGM provided deeper insights into the composition of the water microbiome. The NT Mycobacteria was the most abundant genus present in all water samples among clinically relevant bacteria.

	Relative abundance %																							
	1			2			3			4			5			6			7			8		
Microorganisms	16S	16S-23S	SMG	16S	16S-23S	SMG	16S	16S-23S	SMG	16S	16S-23S	SMG	16S	16S-23S	SMG	16S	16S-23S	SMG	16S	16S-23S	SMG	16S	16S-23S	SMG
Mycobacterium spp.	< 1%	< 1%	1.20%	< 1%	< 1%	7.20%	< 1%	< 1%	12%	< 1%	< 1%	27%	< 1%	< 1%	3.60%	< 1%	< 1%	17%	< 1%	1.21%	15%	< 1%	< 1%	21%
Mycobacterium gordonae	< 1%	< 1%	< 1%	< 1%	< 1%	2.40%	< 1%	< 1%	1.40%	< 1%	< 1%	1.70%	< 1%	< 1%	< 1%	< 1%	< 1%	1.40%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%
Legionella spp.	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%
Burkholderia spp.	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	1.10%	< 1%	< 1%	1.90%	< 1%	< 1%	1.3%	< 1%	< 1%	1.60%	< 1%	< 1%	8.3%
Pseudomonas spp.	< 1%	< 1%	1.10%	< 1%	< 1%	1.30%	< 1%	< 1%	< 1%	< 1%	< 1%	1.10%	< 1%	< 1%	1.10%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%
Leptospira spp.	< 1%	< 1%	48%	< 1%	< 1%	3.80%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	4.50%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%

Table: Bacterial relative abundance (%) from SGM calculated as read counts for a specific microorganism / total read counts of classical reads, and from AmpBS data calculated as read counts for a specific microorganism / total number of reads in OTUs.

Indicators or pathogens - Which parameters are suitable for a microbiological risk assessment of the raw water?

Hartmut WIllmitzer
Thuringian Water Administration

For the risk assessment of water recovery systems, parameters must be used which allow conclusions to be drawn regarding the presence of pathogens and, on the other hand, also occur in a corresponding statistically assured quantity. Current findings show that the parameters investigated so far for the indication in drinking water are suitable as indicators in the raw water to characterize the respective measuring points. However, there are no correlations regarding the concentration of indicator bacteria and pathogens.

In the natural raw water from lakes and reservoirs live about 10 million bacteria per milliliter. These are not all monitored because they are not pathogenic. Also drinking water is not sterile. It is crucial that evidence is provided that the parameters that are representative of pathogens are not contained in the drinking water. Then it is also ensured that no resistant pathogens are in the water.

Impact of wastewater discharges on the resistome and the mobilome of surface waters (the Saale River and the Ter River as model systems)

Carles M. Borrego[1,2], José Luis Balcázar[1], Itziar Lekunberri[1], Alexandre Sànchez-Melsió[1], Marta Villagrasa[1], Bernd Giese[3], Jens Müller[3]

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Wastewater discharges introduce antibiotic residues and antibiotic-resistant bacteria (ARB) into surface waters. Both inputs directly affect the aquatic resistome, either by exerting a selective pressure that favor the proliferation of resistant phenotypes or by enriching the resident communities with wastewater-associated ARB. Here, we investigated the occurrence and persistence of antibiotic resistance genes (ARGs) in environmental samples collected upstream and downstream of WWTP discharge points in the Saale River (Germany) at four different seasons, as well as in the Ter River (Spain). We investigated if this anthropogenic pollution also affects the abundance and diversity of mobile genetic elements (MGEs), which play a crucial role in the spread of antibiotic resistance among bacteria.

Quantitative PCR was used to assess the abundance of genes encoding resistance to β-lactams (bla_{TEM}, bla_{KPC} and bla_{CTX}), fluoroquinolones (*qnrS*), glycopeptide antibiotics (*vanA*), macrolides (*ermB*), sulfonamides (*sul1*), and tetracyclines (*tetW, tetO* and *tetM*). Our results corroborated that WWTP discharges promote the persistence and spread of antibiotic resistance in microbial communities, with a clear seasonal trend in abundance in the Saale River and a significant effect of the collection site (upstream *vs.* downstream) in river Ter. In both rivers, however, the concentration of antibiotic residues in the flowing water were far below the predicted concentrations that select for resistance (Ciprofloxacin in river Ter being the exception). These results suggest that ARB released into the stream by WWTP effluents probably have a larger effect on the river resistome than antibiotic pollution although the latter may contribute to maintain the resistant phylotypes in the system.

In the Ter River, we also analyzed the abundance of ARGs in plasmid and phage DNA fractions to assess the impact of WWTP discharges on these MGE. Interestingly, genes conferring resistance to β-lactams and glycopeptide antibiotics showed significant differences ($p<0.05$) between upstream and downstream sites in both plasmid and phage DNA but no statistical significance was obtained for the same genes in the bacterial DNA fraction. Similar results were obtained using metagenomics, which revealed significant differences in the abundance of ARGs and MGEs (*i.e.* integron and phage integrases and insertion sequences) between upstream and downstream sites. Altogether, our results suggest that WWTP discharges not only have an effect on the abundance and diversity of ARGs but they may also favor their dissemination among aquatic microorganisms.

Human exposure to antibiotic resistant *Escherichia coli* through drinking and irrigation water sources

E. O'Flaherty[1], C.M. Borrego[2 3], J.L. Balcázar[2], A. Solimini[4], F. Pantanella[4], E. Cummins
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[4]Department of Public Health, Sapienza University of Rome, Italy.

Antibiotic resistant infections are a serious threat to public health and when contracted by humans can lead to serious illness or death due to the complications associated with these types of infections. Research shows that many antibiotic resistant bacteria are not efficiently removed during conventional water treatment processes and thus they are discharged into surface waters around the world. This study examines the potential human exposure to antibiotic resistant *Escherichia coli (*AR *E. coli)* through the consumption of tap water while a framework model examining the potential human exposure to AR *E. coli* from irrigated crops is also presented. Water samples were collected from a river located near a drinking water treatment plant and from a river that is used to irrigate local crops, and further analyzed for the presence of AR *E. coli*. A scientific literature search was performed for both models where data on the effect of environmental conditions on AR *E. coli*, the effect of different water treatments on AR *E. coli* concentrations and the amount of human tap water consumed were collected for the drinking water model. Data on the attachment, survival and accumulation of AR *E. coli* on crops after irrigation, the effect of post-harvest treatments, the effect of consumer washing and the human consumption of the crop were collected for the irrigation framework model. The models were created using Microsoft Excel 2013 with the @Risk 7.5 add on (Palisade Corporation, Newfield NY) and Monte Carlo Simulation was performed using probability distributions to characterise uncertainty and variability in the model input data to generate the output distributions. Results are presented for the drinking water model while a framework is presented for the irrigation model as developments of this model are ongoing. The results from the drinking water model show the mean exposure to AR *E. coli* through drinking tap water for an adult varied between 3.44×10^{-7} and 2.95×10^{-1}cfu/day relative to the type and combinations of water treatment used. The level of AR *E. coli* required in the source water to exceed the EU Drinking Water Directive was between 1 and 5 log cfu/ml depending on the water treatment combination used. The results also showed that using a combination of coagulation, flocculation, sedimentation, sand filtration and UV gave the largest reduction of human exposure to AR *E. coli*. This model provides important data on potential human exposure to ARB through drinking water, it identifies the most suitable types and combinations of water treatments to reduce human exposure and it helps to quantify the level of ARB required in a source water supply for drinking water contamination. This type of information could be used to help define monitoring criteria examining the potential exposure to AR *E. coli* through tap water.

The Analysis of Water, Food, and Antibiotics in a Commercial Laboratory

Jens Müller, Annette Pohl, Bernd Giese
Synlab Umweltinstitut GmbH, Lebensmittelinstitut Jena, Orlaweg 2, D-07743 Jena

The SYNLAB group is a world leading laboratory services provider with footprints in over 40 countries. It combines expertise in the areas of Clinical Medicine, Veterinary Medicine, Environment, Food, Hygiene, Pharma, and Tribology. A short overview over the service portfolio in the areas food and water analyses will be given. SYNLAB in general and the SYNLAB Food Institute Jena strive to be one stop shops for their customers. At the Jena site a broad range of services in the fields of microbiology, food chemistry, residues and contaminants, feedingstuffs, molecular biology, and water are offered.
Typical results of the analyses for antibiotic residues, antibiotics resistant bacteria, and antibiotic resistant genes will be presented and discussed.
Compared to other residues and contaminants like pesticides, dioxins, mycotoxins or heavy metals, the determination of antibiotics in food is demanded very rarely. Among a multitude of antibiotic resistant germs, **M**ethicillin-**R**esistant **S**taphylococcus **A**ureus (MRSA) and **E**xtended **S**pectrum **B**eta-**L**actamase producing bacteria (ESBL) are the only ones tested for in food at least occasionally. The detection or the identification of **A**ntibiotic **R**esistance **G**enes (ARG) is not yet been asked for within routine food and water control, but only within specific R&D projects.

Antibiotics and resistance in waste water

Peter Krebs
Technical University Dresden

Antibiotika werden verschrieben, konsumiert, ausgeschieden und ins Abwassersystem eingetragen. Da verhalten sie sich gänzlich unterschiedlich: einige werden mit dem Abwasser transportiert, andere adsorbieren an Oberflächen von Partikeln und werden so in der Kanalisation zurückgehalten bzw. lagern sich in den belebten Schlamm ein, wiederum andere werden in der Kanalisation oder in der Kläranlage abgebaut, wobei unterschiedlich kritische Metabolite entstehen können. Unter Einbezug verfügbarer Informationen und eigener Versuche wurde ein Stoffflussmodell entwickelt, das Stofffrachten und –konzentrationen von der Verschreibung bis zum Fließgewässer beschreibt.
Die Möglichkeit zur Vorhersage erhöhter Antibiotikaverschreibungen über eine Korrelation mit Google flu trends konnte nachgewiesen werden. Leider wurde diese Information vom Netz genommen.
Im Vortrag werden zudem Untersuchungen zum Eintrag und zur Entwicklung von Antibiotikaresistenz *in die* bzw. *in der* Kläranlage erläutert. Es zeigt sich, dass die Anteile resistenter und multiresistenter Mikroorganismen in der biologischen Stufe zunehmen. Die weitere Reduktion von Feststoffen im Ablauf der Kläranlage erhält somit eine besondere Bedeutung.

A simple spectrophotometer for colorimetric DNA assays

Matthias Urban, Ondrej Stranik and Wolfgang Fritzsche
Leibniz Institute of Photonic Technology (IPHT), Department Nanobiophotonics, Jena, Germany

Colorimetric molecular assays (e.g. for the detection of DNA) require just a detection of a significant color change, usually from red (separated gold nanopartciles) to blue (aggregated particles). In order to enable on-site measurements also in less developed regions, a simple LED photometer would be sufficient, The device has to be simple, cost-efficient, robust and easy to replicate. It must be modular and therefore open to modifications during assay development.

We present here such an development of an LED based photometer. A beam splitter is utilized in order to enable a two wavelength measurement with high precision. The sensors for blank and sample measures texactly at the same time, allowing for full compensation of any change in the light source. So the lightness of the light source (LED) must not stabilized.The device comprises three sample ports, enabling measurements in up to 6 wavelength.

In a first test we successfully monitored the color change from Au nanoparticles (20 nm) during their aggregation after adding salt, which overcomes the colloidal stabilization.

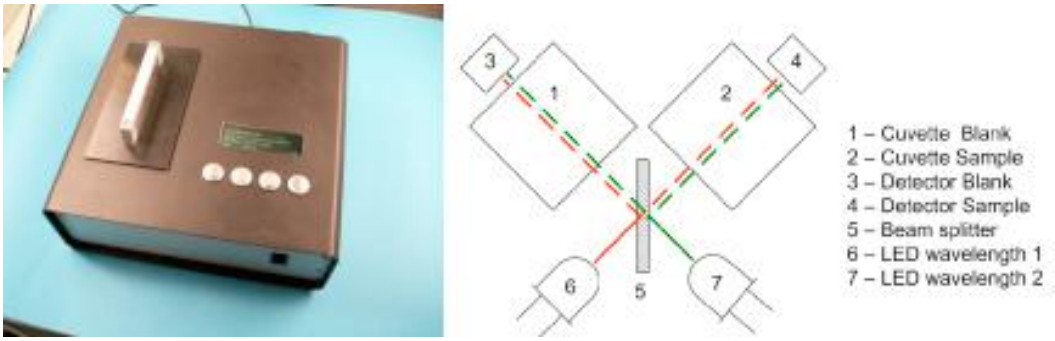

Fig. 1: Photo of the instrument showig the closed sample loading port as well as the display (left). The scheme describes how the light is split into two beams, allowing for a simultaneous measurment of sample and blank.

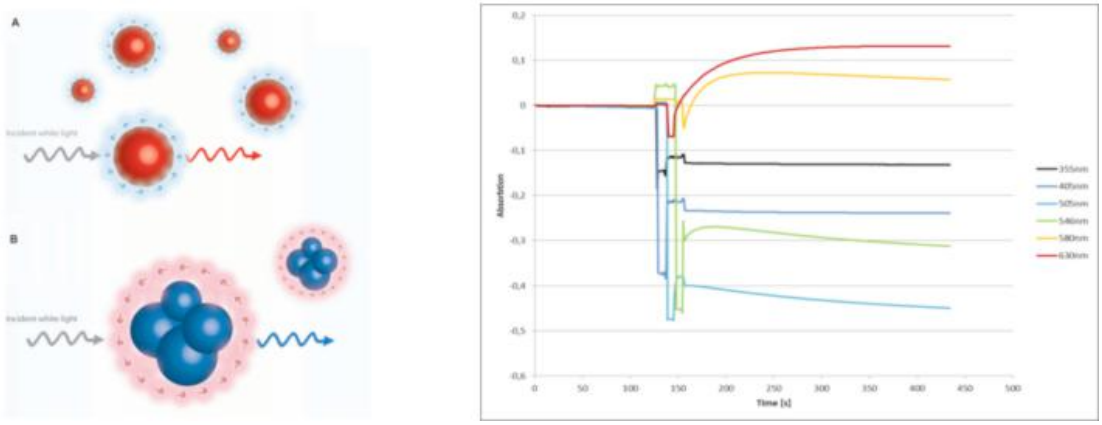

Fig. 2: Test of the instrument: The aggregation of gold nanoparticles was monitored, witha color change from red to bluish. At a certain point, salt is added, then the various colors change accordingly.

Parallel identification of DNA by localized surface plasmon resonance (LSPR) by an imaging spectrometer

David Zopf, Jacqueline Jatschka, Sophie Thamm, André Dathe, Andrea Csáki, Wolfgang Fritzsche and Ondrej Stranik

Leibniz Institute of Photonic Technology (IPHT), Department Nanobiophotonics, Jena, Germany

We present a Fourier transform imaging spectrometer for the parallel read-out of sensors based on noble metallic nanoparticles supporting localized surface plasmons, which are oscillations of the particles' conduction electrons and can be excited by a free propagating light beam. The localized surface plasmon resonance (LSPR) frequency, which depends on the nanoparticles' size, shape, material and the local dielectric environment, manifests itself as a peak in the extinction and scattering spectrum of the particle. The sensor principle is based on the sensitivity of the scattering/extinction spectra upon changes of the local refractive index around the nanoparticle, such changes will be induced upon binding of molecules. The spectroscopy of metal nanoparticles shows great potential for label-free sensing. In combination with a microfluidic system our hyper-spectral imaging system allows the full spectroscopic characterization of many individual nanoparticles simultaneously. We experimentally quantified (incorporating atomic force microscopy as well) the correlation between geometry, position of plasmon resonance and sensitivity of the particles. We were able to follow the adsorption of protein layers and determined their spatial inhomogeneity with the help of hyperspectral imaging of single nanoparticles.

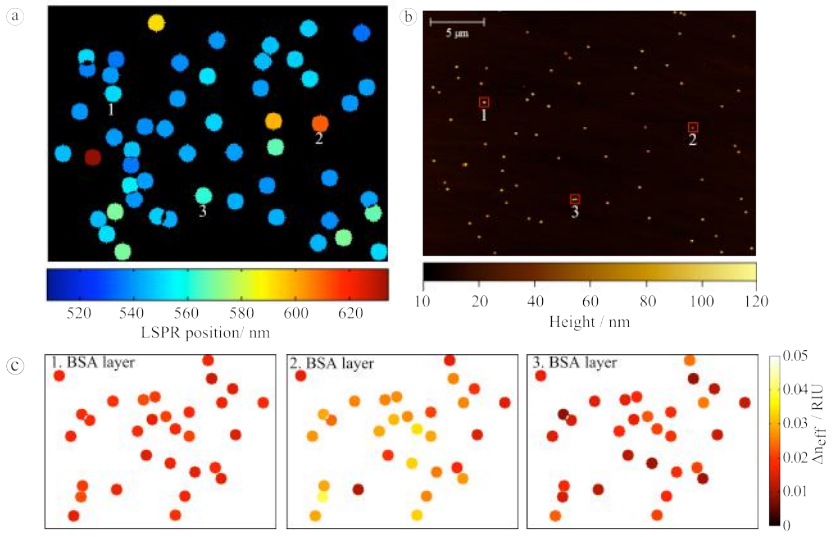

Fig. 1: (a) Lateral distribution of the elected particle ensemble, each particle is color coded according to its LSPR peak wavelength. (b) AFM-image of the region of interest shown in a). (c) False color image displaying the change of the effective refractive index on 30 single nanoparticles induced by the successive adsorption of BSA layers [1].

*[1] D. Zopf, J. Jatschka, A. Dathe, N. Jahr, W. Fritzsche and O. Stranik, Biosensors and Bioelectronics, 2016, **81**, 287.*