

Research and Innovation Conference

Interface of Engineering & Biology

PROGRAM

Introduction by Track Chairs	Yuris Dzenis , <i>Professor, Engineering Mechanics</i> Sally Mackenzie , <i>Professor, School of Biological Sciences</i>
1:30-2:00	Yuri Lyubchenko <i>"Nucleosome dynamics - single molecule AFM study"</i>
2:00-2:30	Richard Gursky <i>"Tools for Biological Electron Tomography, What's New and What Can it do for my Research"</i>
2:30-3:00	Taylor Cavanah and Guido Verbeck <i>"The Intersection of Nanoprobing and Biology"</i>
Break	
3:00-3:10	
3:10-3:40	Tanjef Szellas <i>"Super resolution MICROSCOPY beyond the Abbe limit – STED & other concepts to break the diffraction barrier"</i>
3:40-4:10	Karin van Dijk <i>"Genome-wide map of histone H3 lysine 4 methylation under stress conditions in Arabidopsis thaliana"</i>
4:10-4:40	Bin Yu <i>"MicroRNA methylation in Arabidopsis thaliana"</i>

ABSTRACTS

"Nucleosome dynamics - single molecule AFM study"

The dynamics of nucleosomes is an important property of these fundamental units of the chromatin enabling the access to the DNA by polymerases and regulatory proteins. The mechanism whereby the nucleosome provide this access and the scale of the opening of the nucleosome remain unknown. We applied single molecule time lapse AFM imaging to visualize directly the dynamics of nucleosome and identify the mechanism of the DNA exposure. With this technique we are able to observe a large scale opening of the nucleosomes in which the DNA is unwrapping from the histone core. The unwrapping occurs from the ends of the nucleosome leading to exposing of large DNA regions. The process of the nucleosome occurs in two steps. In the first step the initially tightly wrapped nucleosome releases DNA in various fashions starting from the fast unwrapping to a slow unwrapping process with gradually decreasing number of DNA wrapped around the core. In both cases a transient state with one DNA turn around the core is formed. The second step is the dissociation of this transient nucleosome state that occurs abruptly with no detectable transient states. The unwrapping process can be accompanied by the release of histones, but the dissociation of the core is not required, so the DNA can fully unwrap without the change of the histone octamer size.

"Tools for Biological Electron Tomography, What's New and What Can it do for my Research"

Electron Tomography (ET) had traditionally taken hours of tedious Data collection and weeks of heavy computing power to produce a single Tomogram (reconstructed 3D volume). With the arrival of fast and cheap computing power (PCs) and the development

of automated Instruments, a meaningful Tomogram can be produced in just a few hours. Now that High Resolution Electron Tomography is available to everyone, the questions that should be asked is what will it do for me and how can it answer questions that I have that Light microscopy can not answer? Has it really become easy and fast enough that anyone can accomplish ET successfully and what are the correct tools for my research needs?

"The Intersection of Nanoprobing and Biology"

Nanomanipulation has been gaining recognition over the last ten years due to a success in electrically and mechanically characterizing nanostructures. Beginning five years ago the technology has been used to perform failure analysis in the microelectronics industry. We see the bionano industry as the next promising market. Initial work has been done in cellular nanosurgery, nanoinjection, intracellular pH sensing, and electrical probing. Dr. Verbeck will present his most recent work on the analysis of selected lipid bodies from a cell using nanomanipulation coupled directly to nanospray mass spectrometry. This new method gives us the ability to see the composition of a selected lipid body compared to the field. This process develops direct cell sampling, allowing for analysis of the cells function and role in an organism. This tool set also allows for further study in micro-phase extraction. The nanomanipulator couples the microscope to a virtual-reality interface that gives the scientist virtual telepresence on the surface, scaled by a factor of about a million to one. It provides new ways of interacting with materials and objects at the nanometer scale, placing the scientist on the surface, in control, while an experiment is happening. Applied to micro-phases extraction, the desired interface can be used to concentrate lipophilic and non-polar organics within aqueous media.

"Super resolution MICROSCOPY beyond the Abbe limit – STED & other concepts to break the diffraction barrier"

The groundbreaking concept of stimulated emission depletion (STED), invented by Prof. Stefan Hell [1], has allowed elevating light microscopy beyond established limits. It has resulted in answering many biological questions [2,3,4,5], already, although the access to this complex technology has been restricted to a limited group of experienced researchers, so far. Leica Microsystems has developed a fully integrated super resolution system for the daily research – based on the confocal platform TCS SP5. The general concept STED microscopy will be introduced. The technical realization of the TCS STED is presented. An introduction into the workflow will be given. No compromise has been made on the flexibility of the TCS SP5 giving access to e.g. multicolor recordings in combination with STED imaging and the full capability of optical sectioning to generate 3D data sets. Quasi video rate STED imaging is demonstrated to underline the usability of STED even for fast imaging. Methods to perform live cell microscopy based on STED are discussed. Examples from different field of applications are presented, such as physiology, neuroscience and others to show the usability for many different fields of research.

"Genome-wide map of histone H3 lysine 4 methylation under stress conditions in Arabidopsis thaliana"

Gene-specific transcription requires sequence-specific binding of regulatory molecules to DNA in chromatin. These specific interactions are influenced by the complex chemical modifications to the nucleosomes, DNA and associated proteins that collectively form the chromatin. The biological effects of these chromatin modifications and their recognition by chromatin regulatory or remodeling proteins to modulate transcription are still being established. One of the nucleosome modifications, Histone H3 Lysine 4 (H3K4) methylation, has generally been associated with actively transcribed genomic regions. To investigate the effect of changes in environmental conditions on changes in transcription and associated chromatin modifi-

cations we analyzed the effect of water deficit stress in *Arabidopsis thaliana* on genome transcription and the distribution of histone H3 lysine 4 (H3K4) mono-, di- and tri-methylation. Nucleosome-containing DNA was immunoprecipitated using antibodies specific for each modification and analyzed using high throughput DNA sequencing. The percentage of H3K4 mono-, di- or tri-methylated peaks that were closely associated with known genes ranged from 86 to 96%. Additionally, 92% of all genes were marked by one or more types of H3K4 methylation, predominantly with sharp transitions at their 5' and 3' termini. Only 1% of genes had detectable expression levels and lacked H3K4 mono-, di- or tri-methylation. All of the 636 genes that increased their transcript levels by at least four-fold in response to water deficit stress were found to have increased H3K4me3 levels.

"MicroRNA methylation in *Arabidopsis thaliana*"

MicroRNAs (miRNAs) are 20 to 24 nucleotide (nt) RNAs that function as sequence-specific regulators of gene expression through target cleavage and translational inhibition. We have demonstrated that the 2'-OH of the 3'-terminal nucleotide of miRNAs is methylated in *Arabidopsis* by a methyltransferase, HUA ENHANCER1 (HEN1). In plants carrying a hen1 mutation, small RNAs lack methylation and become reduced in abundance and heterogeneity in size. The size heterogeneity is due to the presence of 1-6 additional nucleotides, usually uridines, at the 3' end of miRNAs. Therefore small RNA methylation protects small RNA from degradation and uridylation. The nature of the enzymes with these activities is currently unknown. A genetic screen for enzymes targeting unmethylated miRNAs or regulators of HEN1 identified two subunits of the plant-specific, putative DNA dependent RNA polymerase IVa (pol IVa), which is essential for the biogenesis of 24 nt endogenous siRNAs. A mutation in RNA dependent RNA polymerase 2 (RDR2), another essential gene for the biogenesis of endogenous 24 nt siRNAs, also rescued the defects in miRNA methylation of hen1-2, revealing a previously unsuspected, negative influence of siRNAs in HEN1-mediated miRNA methylation.

SPEAKER BIOS

Yuri Lyubchenko, Ph.D., D.Sc., Professor, University of Nebraska Medical Center. He earned his PhD from the Moscow Institute of Physics and Technology, Moscow Russia and DSc degree in Molecular biology from Institute of Molecular Genetics, Moscow Russia. He is a biophysicist working in various areas of molecular biophysics including (i) structure of DNA in relation to replication and recombination, (ii) structure of site specific protein-DNA complexes and (iii) structure and dynamics of chromatin and (iv) protein folding biophysics with a major emphasis on understanding protein misfolding phenomena. He published more than 160 papers in a variety of journals and edited books including ranking journals such as Nature and Proceedings of National Academy of Sciences. He applies an arsenal of single molecule biophysics techniques such as an atomic force microscopy (AFM) for topographic imaging including time-lapse detection of molecular dynamics and interactions, single molecule fluorescence microscopy including FRET and AFM force spectroscopy. Development of collateral surface chemistries is an important technological area of Lyubchenko's research enabling him to apply single molecule methods within the broader field of single molecule biophysics.

Richard Gursky studied under Dr. M.A. Hayat, at Kean College of NJ where he received a BA in Biology in 1979. Richard has worked in Electron Microscopy for the past 30 years, using this and other tools in Research and Development for Celanese Research, Mobile Research and Unilever Research. Most recently Richard worked in fundamental Biological research of macro-molecular machines with the Howard Hughes Medical Institute, where he also evaluated

automation SW for computer aided morphological data acquisition for both single particles and for Electron Tomography. Working at FEI for the past 2.5 years, Richard's current responsibilities include Tecnai and Polara customer training, interfacing with the Bio / Cryo / Structural Bio community and FEI technical sales support.

Taylor Cavanah joined Zyvex in May 2004. He currently leads the business development efforts for Zyvex Instruments. His position reflects Zyvex's commitment to expanding their leadership position in the industry. He was previously Zyvex's NanoWorks® Tools Product Manager where he rationalized the product line and repositioned the products for better alignment with the semiconductor industry. Taylor also developed a product roadmap and a detailed market report on the nanoprobe industry. During his tenure as an Applications Development Scientist at Zyvex, Taylor worked to develop the semiconductor IC probing application, as well as nanostructure probing techniques.

Guido F. Verbeck, Assistant Professor of Chemistry, is an expert in mass spectrometry, specifically instrument design and development. Dr. Verbeck received his PhD as a Proctor & Gamble fellow in chemistry under Dr. David H. Russell at Texas A&M University. Dr. Verbeck has developed ion cyclotron resonance, time-of-flight, and ion trap mass spectrometers over the past 10 years, and has been a member of the analytical community for 15 years. Among this design portfolio, Dr. Verbeck has developed a miniature ion trap mass spectrometer at Oak Ridge National Laboratory. Dr. Verbeck's appointment is at the University of North Texas where he continues to design novel ion optical devices for miniaturization and preparative mass spectrometry.

Tan Jef Szellas is the global Product Manager for the Super resolution Technologies, namely 4PI and STED, at Leica Microsystems CMS with global headquarters in Wetzlar, Germany. Leica Microsystems is a leading global designer and producer of innovative, high-tech, precision optical systems for the analysis of microstructures as well as a broad product portfolio for histopathology. Tan Jef graduated in 1999 in biochemistry. He received his PhD from the Max-Planck Institute for Biophysics (Frankfurt/Germany) in 2002, working in the field of electrophysiology. Between 2002 and 2004 he worked as a Postdoc at the neurophysiological department of the University of Goettingen. In 2004 Tan Jef joined Leica Microsystems as an application specialist 4PI Confocal Microscopy and in 2005 he extended his responsibilities to the global product management of 4PI microscopy. In 2007 he became the global product manager of the STED microscope, invented by Prof. Stefan Hell from the Max Planck Institute in Goettingen.

Karin van Dijk is an Assistant Professor in the Biology Department at Creighton University. She earned her PhD from the Department of Plant Pathology at Cornell University. Prior to joining Creighton University, Dr. van Dijk held several Postdoctoral Research positions with a research focus ranging from host-pathogen interactions to epigenetic processes in *Chlamydomonas* and *Arabidopsis*. She has been a co-author on several book chapters and has published her research in a variety of journals including the Proceedings of the National Academy of Sciences, the Journal of Bacteriology, the Plant Cell, Molecular Microbiology, Genetics and Nucleic Acid Research. Her current research is focused on the type III secretion system of bacterial pathogens.

Bin Yu is an assistant professor in the School of Biological Sciences & the Center for Plant Science Innovation at UNL. Dr. Yu obtained his Ph.D degree in genetics in 2004 under the supervision of Dr. Christoph Benning at Michigan State University. From 2004-2008, he worked on microRNA biogenesis as a postdoc in Dr. Xuemei Chen's Lab at the University of California, Riverside, where he uncovered methylation as a novel step of miRNA biogenesis. In 2008, Dr. Yu joined UNL, where he continues research on dissecting the molecular mechanisms regulating microRNA biogenesis and function.