01:30 Welcome & Introduction
  Emmanuel Paris, Boume Boudjelida, Klaus Pross

01:45 The new BioAFM World: From Tissue Characterization to Electrochemical Pore Monitoring and High-Speed Imaging of Molecular Kinetics
  Heiko Haschke, Bruker BioAFM, Berlin, Germany

Session 1: Mechanobiology

02:00 Cell Surface Mechanics gate Embryonic Stem Cell Differentiation
  Alba Diz-Muñoz, EMBL, Heidelberg, Germany

02:15 Mechanobiology and Gynecological Cancers
  Lewis Francis, Swansea University Medical School, UK

02:30 Live Lab Demo: New BioAFM Cell Mechanics Capabilities on the NanoWizard® XP
  Andre Koernig, Bruker BioAFM, Berlin, Germany
  Q&A

03:00 Coffee Break & Game

Session 2: Single Molecule & High-Speed AFM

03:10 Imaging Molecules in Soft Matter and Biology
  Nic Mullin, University of Sheffield, UK

03:25 AFM as a Tool for the Characterization of Lipid-Lipid Interactions in Bilayers containing Polar Sphingolipids, Ceramide and Cholesterol
  Emilio J. González-Ramírez, BIOFISIKA Institute, Bilbao, Spain

03:40 Live Lab Demo: Single Molecule High-Speed Imaging with the new NanoRacer® HS-AFM
  Andreas Kraus, Bruker BioAFM, Berlin, Germany
  Q&A

04:10 Meet your Local Team: Languages - French, German and English.
  Quiz: Win a 1500€ Cantilever Voucher!

04:30 Photothermal AFM-IR Spectroscopy and Imaging: When AFM meets Infrared
  Alex Dazzi, University Paris-Saclay, France

04:50 Closing & Award Ceremony
  Emmanuel Paris, Boume Boudjelida, Klaus Pross

05:00 End
Abstracts and Presenters

Cell Surface Mechanics Gate Embryonic Stem Cell Differentiation

Dr Alba Diz-Muñoz, EMBL, Heidelberg, Germany

Cell differentiation typically occurs with concomitant shape transitions which enable specialized functions. In order to adopt a different shape, cells need to change the mechanical properties of their surface. However, whether cell surface mechanics control the process of differentiation has been relatively unexplored. Here, we show that membrane mechanics gate the exit of mouse embryonic stem cells from naive pluripotency. By measuring membrane tension during early differentiation, we find that naive stem cells release their plasma membrane from the underlying actin cortex when transitioning to a primed state. By mechanically tethering the plasma membrane to the cortex by enhancing Ezrin activity or expressing a synthetic signaling-inert linker, we demonstrate that preventing this detachment forces stem cells to retain their naive pluripotent identity. We have thus identified a decrease in membrane-to-cortex attachment to be a new cell-intrinsic mechanism that is essential for stem cells to exit pluripotency.

Alba Diz-Muñoz received her PhD at the Max Planck Institute for Cell Biology and Genetics in Dresden, Germany. She did her postdoctoral research at UC Berkeley and UCSF, USA. In 2016, Alba established her laboratory at EMBL Heidelberg on Mechanobiology of the Cell Surface. Her group studies the crosstalk between the mechanical properties of the cell surface and the extracellular environment and intracellular signaling processes that drive morphogenesis and fate in animal cells.

Mechanobiology and Gynaecological Cancers

Lewis Francis, Swansea University Medical School, UK

Ovarian Cancer (OC) is the most deadly gynaecological cancer, it is associated with resistance, relapse and a low five year survival rate. OC is highly heterogenous and aggressive, presenting distinct disease phenotypes linked to cell invasion and metastasis. OC cell invasion and metastasis are associated with changes in cellular and spheroid mechanical properties that are brought about by complex activation/inactivation of multiple signalling pathways and (epi)genomic processes. Our lab is developing new and improved, multimetric approaches to investigating OC cell mechanobiology in 2D and 3D patient derived cultures. Using the multiple analysis modes of Atomic Force Microscopy, alongside epigenomic manipulations and immunofluorescence analysis, we hope to reveal disease specific mechanical phenotypes that lead to novel targeting strategies and functional genomic approaches. The presentation will focus on AFM applications in 3D cancer space and identifying trends in the nanomechanical properties of spheroid cultures in the presence of epigenetic modifying drugs.
Based at the Medical School, Dr Francis is an Associate Professor in the Reproductive Biology and Gynaecological Oncology Research Group (RBGO) and leads the groups Biophysics and Epigenetics research programmes. Lewis has developed tissue, cell and molecular biochemical and biophysical research approaches for functional cell and molecular analysis in both human reproductive and regenerative medicine. Exploring cellular heterogeneity in complex tissue micro-environments, Lewis’ research aims to identify distinct cell populations for therapeutic targeting through advanced drug delivery systems. By adopting disease specific phenotype models in both cell line and patient derived systems, his research focuses on the exploration of biomarker expression, gene regulation networks and cell-matrix interactions in niche tissue spaces. Lewis is also the Programme Director for the Nanomedicine MSc, Medical School Director of ACNM, and Deputy Director of the CALIN project.

Imaging molecules in Soft Matter and Biology

**Nic Mullin, University of Sheffield, Sheffield, UK**

Soft matter and biological systems are extremely sensitive to applied force. In this talk, I will briefly discuss strategies for imaging at low force while maintaining a high signal-to-noise ratio in dynamic AFM, and present recent work carried out on synthetic polymers, biological membranes and bacteria.

**Dr Nic Mullin received his PhD in method development for scanning probe microscopy in 2009 from the University of Sheffield. He worked as a postdoc on various multidisciplinary projects involving AFM until 2015, when he started his current position as a Senior Experimental Officer in the Biophysical Imaging Centre at the University of Sheffield. He splits his time between applications of AFM, training and support of their lab users and scanning probe instrument development projects.**
AFM as a Tool for the Characterization of Lipid-Lipid Interactions in Bilayers containing Polar Sphingolipids, Ceramide and Cholesterol

Emilio J. González-Ramírez, BIOFISIKA Institute, Bilbao, Spain

Our group uses the atomic force microscopy (AFM) technique to characterize the lipid-lipid interactions present on systems composed of three or four lipid components, among them: One with a high phase transition temperature (Tm), one with a low Tm, and cholesterol. In these studies, AFM is used to identify the different phases formed due to the phase segregation observed in the sample. With force spectroscopy measurements we are able to characterize the different phases using their breakthrough force value (nN), which is related to the force needed to pierce the lipid bilayer. In addition, we also use Rhodamine-PE in the epifluorescence visualization of supported planar bilayers coupled to AFM. Together, these results allow the characterization of the samples.

Dr Emilio J. González-Ramírez is a postdoctoral researcher in Felix Goñi’s Sphingolipids and Membrane Domains Group. He received a doctorate in Molecular Biology and Biochemistry in 2019 at the University of the Basque Country. His doctoral studies were focused on lipid-lipid interactions in model membranes using techniques such as differential scanning calorimetry (DSC), confocal microscopy, and atomic force microscopy (AFM).

Photothermal AFM-IR Spectroscopy and Imaging: When AFM meets Infrared

Alex Dazzi, Institut de Chimie Physique, Université Paris-Saclay, France

The invention and development of the AFM-IR technique began because of a strong wish to go beyond resolution and push the limit of infrared microscopy in the Free Electron Laser Center at Orsay in 2004. The idea of AFM-IR is based on the coupling of a tunable infrared laser and an AFM (Atomic Force Microscope). The sample was irradiated with a pulsed nanosecond tunable laser in total reflection configuration to avoid tip illumination. If the IR laser is tuned to a wavenumber corresponding to sample absorption band, the absorbed light is directly transformed into heat. This fast heating results in a rapid thermal expansion localized only in the absorption region. The thermal expansion is then detected by the AFM tip as a shock as the cantilever will oscillate on its own resonance modes. Because of the damping with the surface contact this oscillation will decrease in function of time (ring down). The 4-quadrants detector of the AFM records these oscillations. Thus, the detection scheme is analogous to photo-acoustic spectroscopy, except that the AFM tip and cantilever are used to detect and amplify the thermal expansion signal instead of a microphone in a gas cell. As oscillations amplitude detected by the AFM is rigorously proportional to the local absorption, recording for one tip position, the oscillations maximum as a function of laser wavenumber allows a local IR absorption
spectra to be built up. This spectra correlates very well with conventional IR absorption spectra collected in FT-IR. In addition, mapping oscillations amplitude versus tip position, for one specific wavenumber, gives a spatially resolved map of IR absorption that can be used to localize specific chemical functions. After 14 years of development and improvement, the AFM-IR technique has now become a robust and efficient tool for infrared analysis at nanometer scale. The AFM-IR system works in contact or tapping mode with a sensitivity and resolution of around 5-10 nm and a spectra bandwidth of about 0.5 cm\(^{-1}\) (linked to the pulsed laser properties). The range of applications is huge covering diverse research areas like materials science, life science, and astrochemistry.

Alexandre Dazzi is a tenured Professor of Physics at Université Paris-Saclay and works in the Institut de Chimie Physique. His research focuses on the infrared and nanoscience domain. After inventing and developing the AFM-IR technique, he has worked on improving AFM-IR instrumentation and focused on biological applications. He now has a user facility and collaborates with various groups in different domains like astrophysics, culture heritage, polymer science, and microbiology. He was the 2009 laureate for France’s national instrumentation prize from the Société Française Division de Chimie Physique and received the Ernst Abbe Award in 2014 from the New York Microscopical Society.

Please don’t hesitate to contact us at events.bioafm@bruker.com if you have any questions.