A mechano-optical microscope for studying force transduction in living cells

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1. Current State of Research in the Field:

The nanomechanical properties of cells play an important role in many biological processes including cell differentiation, proliferation, and tissue organization. This largely derives from a complex network of cytoarchitectural elements that act in harmony to exert forces, resist compression, and to respond to microenvironmental changes[1-3]. Force transduction pathways span integrins that bind the extracellular matrix, to focal adhesions and cytoskeletal elements such as actin, microtubules and intermediate filaments, to nuclear membrane proteins such as the LINC complex that transduce nanomechanical signals into the nucleus, as one mechanically coupled system[4]. This can in turn modulate nuclear transcriptional programs leading to differentiation of various cell types[5].

The atomic force microscope (AFM) has emerged as a powerful tool to quantify cellular nanomechanics at the cellular and molecular level[6] (Fig. 1). Nevertheless, in the context of *in vivo* cellular studies, AFM is limited in terms of resolving (i) the biochemical identity of biological structures, (ii) subcellular structures, and (iii) their dynamic responses to external force. To circumvent these limitations, AFM is often combined with fluorescence microscopy to image cellular shape and labeled cellular proteins while making force measurements. However, this typically provides "in-plane" (XY) views of the sample parallel to the surface plane. Yet, the most significant cellular deformations and cytoskeletal rearrangements are aligned perpendicular to the surface plane (XZ). Hence, specific sub-cellular conformational changes along the loading direction can be directly correlated to the applied AFM load by fluorescence imaging in the XZ plane. This will enable us to dissect and assign the mechanical contributions of the intra- and inter-cellular components to mechano-phenotypes of living cells[7].

2. Research Proposal

Here, together with Prof. Ernst Meyer, we will build a "mechano-optical microscope" (MOM) that fully synchronizes AFM and optical measurements of live cells. Previously, *the Lim Lab had developed an atomic force microscope (AFM)-based diagnostic apparatus known as ARTIDIS® ("Automated and Reliable Tissue Diagnostics"; U.S. patent 8,756,711) that correlates local indentation-based stiffness measurements across entire tissues*[8]. *The Meyer lab is a world-leading expert in developing ultra-sensitive AFM instrumentation to study nanoscale friction and nanomechanical forces*[9].

The MOM will fully integrate an AFM onto a spinning disk confocal microscope that was recently acquired by the Lim Lab featuring FRAP (fluorescence recovery after photobleaching) and a laser ablation system. The latter will be used as a nanosurgical tool to cut single cytoskeleton fibers or particular structures of cells[10] to investigate pre-tension based on the recoil and relaxation of those structures. In parallel, we want to understand how forces generated by dynamic (AC) indentation and shear stress are transduced within cells. Subsequent experiments include:

(1) In collaboration with Dr. Marija Plodinec@Dept. Pathology, University Hospital Basel, we will apply a model epithelium (Oertle *et al*, "An *in vitro* epithelium that bears the mechanobiological hallmarks of living tissue", *to be submitted*) to quantify and discriminate how actin, IFs, and microtubules, respectively, contribute to cellular mechanics[11] at the sub-cellular level.

(2) The cytoskeleton spans the entire cell from the plasma membrane to the nucleus to provide structural stability as well as to relay mechanical signals[12, 13]. We will trace how fast and how strongly mechanical forces that transduce from the cell surface induce nuclear deformations.

(3) We will resolve specifically modulated changes to the cytoarchitecture by introducing tumorigenic mutations to actin and IFs (i.e., using GFP constructs via transient transfection[14]) and compare the nanomechanical response of transfected and non-transfected cells in terms of (1) and (2).

(4) Our long term goal is to construct a multi-scale rheological model[15] that correlates cellular mechanics to the interplay between the microenvironment, nucleo/cytoarchitecture, and the protein linkages between them.

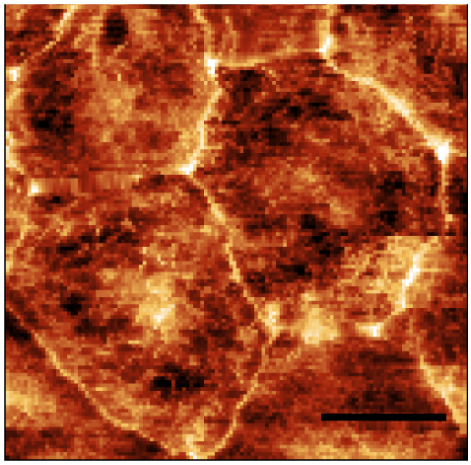

Fig. 1: High-resolution AFM force map reveals subcellular structures in live MDCK cells with intercellular junctions clearly resolved. The image consists of 128 x 128 force curves. Pixel resolution = 240 nm. Scale bar = 10 µm (Oertle *et al.*)

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Education

Ph.D. (Chemical Physics), The National University of Singapore/Institute of Materials Research and Engineering (Singapore) "*Solvation Forces in Confined Molecular Liquids"*, 2003.
B.Sc. in Applied Science (Physics), The University of North Carolina at Chapel Hill, 1996.

Appointments

2014 to pres.	Argovia Professor for Nanobiology (Associate Prof.), Biozentrum and the Swiss Nanoscience Institute, University of Basel, Switzerland
2009 to 2013	Argovia Professor for Nanobiology (Assistant Prof.), Biozentrum and the Swiss Nanoscience Institute, University of Basel, Switzerland
2004 to 2008	Postdoc, NCCR Nanoscale Science, University of Basel, Switzerland

Honors and awards

2008	Pierre-Gilles de Gennes Prize: "From Solid State to Biophysics"
2004	Agency of Science, Technology and Research (Singapore) International Fellowship

Patents

Method for staging cancer progression by atomic force microscopy.
- US patent 8,756,711; issued on 17-June-2014
Method, system and device for controlling a scanning probe microscope.
- European Patent Application EP11193120 Filing date: 12-Dec-2011

Commercial Activities

Co-Inventor, ARTIDIS® (*Automated and Reliable TIssue DIagnostics*)
Co-Founder, Nuomedis® AG (www.nuomedis.com)

Key Publications ('**bold**': corresponding author)

Y. Sakiyama, A. Mazur, L.E. Kapinos and **R.Y.H. Lim**, Spatiotemporal dynamics of the nuclear pore complex transport barrier resolved by high-speed atomic force microscopy. ***Nature Nanotechnology***, advanced online publication (2016). doi: 10.1038/nnano.2016.62

M. Plodinec and **R.Y.H. Lim**, Nanomechanical characterization of living mammary tissues by atomic force microscopy. ***Methods in Molecular Biology***, *1293*, 231-46 (2015)

K.D. Schleicher, S.L. Dettmer, L.E. Kapinos, S. Pagliara, U.F. Keyser, S. Jeney and **R.Y.H. Lim**, Selective transport control on molecular velcro made from intrinsically disordered proteins. ***Nature Nanotechnology*** 9 525 (2014)

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S. W. Kowalczyk, L. Kapinos, T. Magalhães, P. van Nies, **R.Y.H. Lim**, and C. Dekker, Single-molecule transport across an individual biomimetic nuclear pore complex, ***Nature Nanotechnology*** 6, 433 (2011).

Curiculum Vitae of Prof. Dr. Ernst Meyer

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Dr. Ernst Meyer is a Professor of physics. He received his Ph.D. at the University of Basel in 1990. The topic of force microscopy on ionic crystals and layered materials was treated in his thesis. He worked at the IBM Research Center Zurich (1992-1994). In 1997, he started his present position at the University of Basel. His research interests are the development of surface science techniques, such as friction force microscopy and dynamic force microscopy with true atomic resolution. He is also active in the field of nanomechanics, including nanotribology and nanosensors. Further probe microscopes, such as Kelvin probe force microsocpy, scanning spreading resistance microscopy, scanning force capacitance microscopy and dissipation force microscopy are used to probe dopant concentrations of semiconductors or to investigate novel materials, such as graphene or nanodiamonds.

Professional Preparation

University of Basel, Switzerland	Diploma in Physics	M.S. 1987
University of Basel, Switzerland	Condensed Matter Physics	Ph.D. 1992
IBM Research Center Zurich	Nanoscience and Novel Sensors	1992-1994

Appointments

1997 Associate Professor: Department of Physics, University of Basel, Switzerland
2009 Full Professor: Department of Physics, University of Basel, Switzerland

Selected Publications

1. E. Gnecco, R. Bennewitz, T. Gyalog, Ch. Loppacher, M. Bammerlin, E. Meyer, and H.-J. Güntherodt. Velocity Dependence of Atomic Friction. *Phys. Rev. Lett.* **84**, 1172 (2000).

2. Socoliuc, S. Maier, O. Pfeiffer,E. Gnecco, A. Baratoff, R. Bennewitz and E. Meyer, Atomic-Scale Control of Friction by Actuation of Nanometer-Sized Contacts" *Science*, **Vol. 313**, p. 207, July 14 (2006).

3. M. Urbakh and E. Meyer, The renaissance of friction, Nature Materials, 9, 8 (2010).

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273 research papers listed in ISI Web of Knowledge, H-index = 50 with 40 citations per publication on average, More than 100 invited lectures at conferences and invitations to seminars and colloquia, supervisor of more than 20 PhD theses, (co)organizer of 10 international conferences.

Awards and Honors:

2011 MC of the Eurocore Action “UNDERSTANDING AND CONTROLLING NANO AND MESOSCALE FRICTION”
2002 Chairman of the ESF-Programme Nanotribo
2001 Member of the National Center of Competence in Research “Nanoscale Science”
1993 Swiss Physical Society Prize
1992: *venica docendi* at the University of Basel
1990: Dr. Phil., Condensed Matter Physics with *summa cum laude*

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