basic, non-structured head domains with the acidic coiled-coil rods. In a next step, ULFs longitudinally anneal by an end-on-addition mechanism to yield filaments. This mechanism is also exhibited by muscle desmin and the epithelial keratins, whereas the nuclear IF proteins, i.e., the lamins, do not assemble into ULFs. In a next step, the subunit composition of ULFs and IFs of different IF proteins was analyzed by scanning transmission electron microscopy (STEM) and cryo-electron tomography of native specimens. Depending on the ionic conditions used for assembly, on average keratin IFs harbor 8, vimentin IFs 16 and desmin IFs 24 coiled-coil dimers per filament cross-section. The formation of ULFs was investigated further by small-angle X-ray scattering (SAXS) and AUC, employing a mutant vimentin variant that is arrested in the ULF state. With these data at hand, we investigated the impact of human disease mutations found in desmin that cause myofibrillar myopathy. Last but not least, we explored the network formation of lamin A and some of its disease variants, which strongly deviates from that of cytoplasmic IFs. These data give a first mechanistic clue how the lamin network provides mechanical stability to the nuclear envelope and nuclear architecture.

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The Role of Central Microtubules in the Beating of Eukaryotic Flagella, Revealed by High-Speed Holographic Microscopy
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Understand the mechanics of the eukaryotic flagellum is a key challenge in biophysics. As well as being of scientific interest, there are clear therapeutic applications, not least in reproductive medicine. The axoneme lies at the heart of the flagellum, and its structure is known: nine microtubule doublets surround a central pair of microtubule singlets. The role of the central pair in this canonical 9+2 arrangement has been the subject of speculation for some time, though they are known to assist in regulating the flagellar beat. Our group has developed high-speed holographic microscope that allows us to numerically refocus a digital image off-line. By generating a stack of refocused images from each frame in a video, we obtain scans of the sample volume at the frame rate of our video camera. Effectively, this allows us to image 500-1000 volumes per second. By analyzing this volumetric data, we can measure the waveform of a eukaryotic flagellum to within 200 nm in three dimensions, and with millisecond time resolution. We have previously used this method to measure the waveform of a naturally occurring, isolated flagellum: the microgamete of the rodent malaria parasite Plasmodium berghei. In order to assess the role of the central pair, we take advantage of a newly-available mutant strain that is lacking one or both central microtubules. By comparing the three-dimensional shape and motion observed in mutant flagella to that in the wild-type, we can get some insight into how the central pair helps to regulate the beating action.

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Impact of Physical Boundaries on the Mechanics of Collagen Gels and Mechanosensation
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Cell adhesion responses to in-depth physical properties such as substrate roughness and topography are well described but little is known about the influence of lateral physical cues such as tissue boundaries on the function of adherent cells. Accordingly, we developed a model system to examine remote cell sensing of lateral boundaries. The model employs floating thin collagen gels supported by rigid grids of varying width. The dynamics, lengths, and numbers of cell extensions were regulated by grid opening size, which in turn determined the distance of cells from rigid physical boundaries. In smaller grids (200 and 500 μm wide), cell-induced deformation fields extended to, and were resisted by, the grid boundaries. However, in larger grids (1700 μm wide), the deformation field did not extend to the grid boundaries, which strongly affected the mean length and number of cell extensions (~60% reduction). The generation of cell extensions in collagen gels required expression of the β1 integrin, focal adhesion kinase and actomyosin activity. We conclude that the presence of physical boundaries interrupts the process of cell-mediated collagen compaction and fiber alignment in the collagen matrix and enhances the formation of cell extensions. This new cell culture platform provides a geometry that more closely approximates the native basement membrane and will help to elucidate the roles of cell extensions and lateral mechanosensing on extracellular matrix remodeling by invasion and degradation.

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Extracting Quantitative Data from AFM Indentations on Soft, Heterogeneous Biomaterials
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Atomic force microscopy (AFM) is a commonly used method for extracting mechanical information about samples ranging from soft biological matter to rigid semiconductors. AFM and other force spectroscopy techniques have been recently employed to examine how the elastic properties of metastatic cancer cells differ from their healthy counterparts and to study the contractile forces that cancer cells exert during the process of invasion through an extracellular matrix (ECM). As the samples being studied with AFM become more complex, novel analysis methods must be developed to produce meaningful and quantitative data, thus new strategies for fitting force-indentation data beyond the standard Hertz model are essential. We present a method of raw data fitting which determines the apparent Young’s modulus as a function of indentation depth, providing sensitivity to sample heterogeneities such as substructural elasticity effects. An improved AFM tip shape model is derived for a spherical apex with a smooth transition to a cone to provide a realistic representation of the experimental AFM tips used. A bonded two-layered elastic model is created to include the perturbations caused by a heterogeneous material, such as a cell embedded in ECM. This model allows for understanding the signal generated from substructure components as well as the theoretical limits for determining the elastic properties of the underlying second layer. To validate this, we performed finite element analysis simulations and AFM indentations on polyacrylamide. We also show specific examples of how these analysis methods and finite element analysis can be employed to extract more information regarding the mechanical basis of cancer cell invasion into an ECM.

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From Rheology to Elasticity: How Can We Capture the Dynamical Properties of Cells?
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Atomic force microscopy has been introduced in the late 1980’s in cellular biology to probe the mechanical response of living systems with a nanoscale resolution. However in traditional force-indentation curves obtained such a tool, the discrimination of elastic, viscous and dynamical aspects of a cell response under mechanical stress is very difficult, because it assumes that we have already designed a correct model to capture this response. Actually the response of a cell span different scales in space and in time. We propose here to revisit the interpretation of force-indentation curves without any a priori model to capture the temporal evolution of the shear modulus of living cells and compare this response to simple visco-elastic models. We show that using multi-scale analyzing tools such as wavelet transforms offers the possibility to survey in real time the different response modes of a cell during an indentation experiment. Because cells are also very different from one tissue to another one, we take cells with different adherence for this discussion; namely strongly adherent cells such as fibroblasts or myoblasts, circulating cells such as blood cells and intermediate cells that have also a very rich metabolic role such as hepatocytes. I acknowledge my collaborators in this work: namely Alain Arneodo, Benjamin Audit, Lotfi Berguiga, Elise Boyer-Provera, Simona Diguini, Guenola Drillon, Bastien Lapenrouzaz, Mael Le Berre, Veronique Maguer Satta, Cristina Martinez-Torres, Mathieu Piel, Laurent Schaeffer and Laura Streppa.

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Cytoskeletal Stiffness Controls the Threshold of T Cell Activation
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T cells utilize a dynamic cytoskeleton to effectively scan antigen-presenting cells and become fully activated. Cytoskeletal activities help the T cell both to survey in real time the different response modes of a cell during an indentation experiment. Because cells are also very different from one tissue to another one, we take cells with different adherence for this discussion; namely strongly adherent cells such as fibroblasts or myoblasts, circulating cells such as blood cells and intermediate cells that have also a very rich metabolic role such as hepatocytes. I acknowledge my collaborators in this work: namely Alain Arneodo, Benjamin Audit, Lotfi Berguiga, Elise Boyer-Provera, Simona Diguini, Guenola Drillon, Bastien Lapenrouzaz, Mael Le Berre, Veronique Maguer Satta, Cristina Martinez-Torres, Mathieu Piel, Laurent Schaeffer and Laura Streppa.