However, the role of keratins for biomechanical properties and invasion of epithelial cells are only partially understood. Here, we address this issue in murine keratinocytes lacking all keratins upon genome engineering. In contrast to prediction, keratin-free cells show an about 60% higher cell deformability even for small deformations. This is compared to less pronounced softening effects for actin depolymerization induced via latrunculin A. To relate these findings with functional consequences, we use invasion and three-dimensional growth assays. These reveal higher invasiveness of keratin-free cells. Re-expression of a small amount of the keratin pair K5/K14 in keratin-free cells reverses the above phenotype for the invasion but does not with respect to cell deformability. Our data shows a novel role of keratins as major player of cell stiffness influencing invasion with implications for epidermal homeostasis and pathogenesis. This study supports the view that downregulation of keratins observed during epithelial-mesenchymal transition directly contributes to the migratory and invasive behavior of tumor cells. (see K. Seltmann et al., PNAS, in press).

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Dry Mass and Cell Cycle Follow-Up from Quantitative Phase Imaging

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During cell cycle, a cell doubles all its components and divides into two cells. Cell cycle is often studied with fluorescent labeling, by flow cytometry. Here, we propose a simple method to analyze cell cycle and dry mass fluctuations using quantitative phase imaging.

The technique is based on a quadri-wave lateral shearing interferometry (QWLSI) wave front sensor [1]. It provides a quantitative measurement of the optical path difference (OPD = $\Delta n \bullet$thickness) in nm. This measurement, when integrated over the cell surface, is directly proportional to the cell dry mass [2], giving direct information on the cell growth. No labeling is needed. It is self-referenced and can be plugged on any microscope with classic objectives, a white light and a camera port. Since it is achromatic, it can be used in near IR for long live cell imaging. It can easily be combined with fluorescence for simultaneous correlative microscopy. Automated segmentation of cells is easy due to the absence of halo or artifacts. It is fast (camera frame-rate limited) and sensitive (diffraction-limited in X and Y, $\pm$ 0.5 nm in OPD, $\pm$ 0.6 pg for a 570 pg cell).

We established criteria integrating both dry mass and morphological parameters to identify different cell cycle stages and growth rate of haploid and diploid yeasts, as well as four different mammalian cell lines, under different conditions. We studied them by time lapse and population snapshot imaging. The method is robust to record cellular division processes and effects of drugs on cell growth.

[1] P Bon, G Maucort, B Wattellier and S Monneret, ''Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells'', Optics Express, 17 (15): 13080-13094 (2009).
[2] R Barer, ''Interference microscopy and mass determination'', Nature, 169: 366-367 (1952).

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How Embryonic Cartilage Grows: Insights Gained from Quantitative Live Imaging

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One central question in development biology is how individual cell behaviors contribute to tissue morphogenesis. Growth plate cartilage contains morphologically distinct populations of chondrocytes in different zones during much of its growth phase, indicating a link between regulated cell behaviors and tissue elongation. To gain insights into this process, we develop a robust avian embryonic metacarpal culture system, and employ time-lapsed live 2-photon laser scanning microscopy to observe the cartilage growth. Quantitative analysis of the cellular displacements during the tissue growth reveals that cells in the proliferative and prehypertrophic zones, though morphologically distinct, display similar displacement trajectories, contributing in a linearly additive fashion to the unidirectional tissue growth. Our analysis rules out cell division and convergence-extension as the driving mechanisms for tissue elongation; rather, anisotropic matrix deposition and cell volume enlargement are responsible for sculpting the directional tissue growth.

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Optical Measurement of Biomechanical Properties of Human Red Blood Cell using Digital Holographic Microscopy: Malaria and Sickle Cell Diseases

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Pathophysiological aspect of several hematologic diseases is largely determined by biomechanical properties of red blood cells (RBCs) and their hemodynamical properties in circulatory system. Here we present the biomechanical properties of individual RBCs from patients with sickle cell disease and RBCs infected with malaria-inducing parasites *Pf. falciparum*. Using laser digital holographic technique, we non-invasively quantify membrane fluctuation in RBCs at the nanometer and millisecond scale, which is analyzed with the mathematical model to retrieve four important mechanical properties of RBCs; bending modulus, shear modulus, area expansion modulus, and cytoplasmic viscosity. We find significant alterations in the mechanical properties of RBCs in several pathophysiological states, ranging from depletion of Adenosine-5'-triphosphate (ATP)[3], different osmotic pressures[4], malaria infections[5,6,7], and sickle cell diseases[9].

References
1. G.A. Barabino, M.O. Platt, and D.K. Kaul, Annual review of biomedical engineering 12, 345 (2010).
2. YK Park, CA Best, K Badizadegan et al., Proceedings of the National Academy of Sciences 107 (15), 6731 (2010).
3. Y. Park, C. A. Best, T. Auth et al., Proceedings of the National Academy of Sciences of the United States of America 107 (4), 1289 (2010).
4. Y. Park, C. A. Best, T. Kuriabova et al., Physical Review E 83 (5) (2011).
5. Y. Park, M. Diez-Silva, G. Popescu et al., Proc. Natl. Acad. Sci. USA 105 (37), 13730 (2008).
6. R. Chandramohanadas, Y. Park, L. Lui et al., PLoS One 6 (6), e20869 (2011).
7. M. Diez-Silva, Y. Park, S. Huang et al., Scientific Reports (2012).
8. H.S. Byun, T.R. Hillman, J.M. Higgins et al., Acta Biomaterialia (2012).
9. Youngchan Kim, John M. Higgins, Ramachandra R. Dasari et al., Journal of Biomedical Optics 17 (4), 0405011 (2012).

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The Nanoscale Organization of Focal Adhesion Signaling Complexes can Reflect Changes in Cellular Contractility and Motility

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Focal adhesions are the conduits through which cells receive and interpret mechanical signals. It is not known if nanoscale protein organization is altered to accommodate changes in mechanical inputs from the cytoskeleton and extracellular matrix components. We hypothesized that the relative position of specific focal adhesion proteins could correspond to the engagement of a physical protein clutch for different adhesion functions. To this end we employed Scanning Angle Interference Microscopy to determine the 3D organization of proteins comprising focal adhesions with a precision of ~5nm. We found paxillin, FAK, vinculin, talin, and zyxin to be stratified in distinct layers over a vertical range of 60 nm. We then compared nascent versus focal adhesions at the cell leading edge, and found that paxillin localized ~7nm towards the cell membrane in developing adhesions. We inhibited intracellular contractility to see how adhesion architecture dynamically responds to changes in mechanical input, and observed that paxillin and zyxin, but not vinculin, undergoes a marked increase in height of >15nm. Conversely, vinculin without a force dependent auto-inhibition domain, T12; undergoes dramatic reorganization at the nanoscale after contractility inhibition. Overexpression of vinculinT12 resulted in increased intramolecular forces as seen in a vinculinT12 FRET tension sensor, targeting of vinculin to an architecture that corresponded with talin and actin engagement, but not changes in cellular traction. When we reduced cellular motility through overexpression of a constitutively active Rac1 mutant, adhesions at the lamella-lamellipodia border had different vinculin architecture than other adhesions in the cell. Our results suggest that elimination of vinculin force dependent auto-inhibition can dictate focal adhesion architecture and