length, aspect ratio, fractal dimension etc. Simultaneously we also use orthogonal polynomial decompositions using Zernike moments to calculate shape characteristics. Using statistical data analysis we compare the ability of the Zernike moment expansion to capture the different shapes and their perturbations with that of the geometric parameters. We find that both types of shape calculations give insights into how a cell determines its shape on a surface.

**695-Pos Board B475**
**Does Cell Shape Determine Cell Fate?**
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Different types of cells, i.e. from different tissues, typically look quite different from each other. Even when cultured on two-dimensional surfaces like glass slides or tissue culture polystyrene under identical conditions, cells adopt different shapes. These shapes are in general functions of the cytoskeletal properties of those cells, itself a subset of what we call the ''state'' of the cell. Moreover the changes in cell shape upon perturbation of the surface or of the cells themselves should reflect their intrinsic cellular properties, i.e. the cell state. Significant evidence has accumulated that changes in shape can also alter cellular properties, at least for some cells. Our experiments suggest that for Mesenchymal Stem Cells (MSCs), shape perturbations have consequences for their differentiation into osteoblasts. Thus shape seemed linked to fate. These statements beg the question: is it possible to use cell shape to assess cell state? For example can we back-calculate the cytoskeletal properties of the cell from the way it looks on surfaces? This question becomes all the more interesting for cancer cells since cancer cells are known to have altered mechanical properties compared to normal cells, and invasive cancer cells appear to have altered mechanical properties compared to non-invasive cancer cells. In this work we present a combination of experiments and statistical data analysis to try to begin to understand how cell shapes are affected by changes in surface properties or by perturbations of the cytoskeleton. We use fluorescent imaging to obtain the two-dimensional profile of cells and novel Third Harmonic Generation methods to obtain three-dimensional images on cells on substrates. We use these experiments to infer how the cell shape of cancer cells could be associated with their invasive properties. We discuss some rudimentary mathematical models based on these results.

**696-Pos Board B476**
**Real-Time Deformability Cytometry: High-Throughput Mechanical Phenotyping for Changes in Cell Function**
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Changes in cell function are often accompanied by alterations of the intrinsic cell structure particularly the cytoskeleton. This leads to distinct mechanical changes. For example, cells become softer during malignant transformation and stiffer during differentiation. Exploiting the mechanical phenotype of cells as an inherent, label-free marker requires a high-throughput and robust measurement technique. Here, we introduce real-time deformability cytometry (RT-DC) for mechanical single cell classification of heterogeneous cell populations at rates of several hundred cells per second in real-time. Performing RT-DC on primary human hematopoietic stem cells and mature blood cells we demonstrate its capability to detect lineage and source specific mechanical phenotypes. We also find that different stages of the cell cycle possess a unique mechanical fingerprint allowing the distinction between cells in G2 and M phase. In summary, RT-DC represents a novel flow cytometric approach that enables the translation of mechanical phenotyping from basic research into applications in biology and medicine.

**697-Pos Board B477**
**Cortical Actin Tension, Elastic Modulus and Cytosolic Pressure in Fibroblasts Determined using Atomic Force Microscopy**
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The cortical actin cytoskeleton lies just beneath the cell membrane to define cell shape and mechanical properties and thus plays a key role in cell biology processes such as migration and morphogenesis. The organization of actin filaments and actomyosin contractility are known to contribute to modifying the mechanical properties of the cortex. However, recent work report how these properties contribute to cortex tension and intracellular pressure. Here we propose a new method for using an atomic force microscope to determine actin cortex mechanical properties of non-adherent human foreskin fibroblasts including the cortex tension and intracellular pressure, but additionally, the cortex elastic modulus which has not been measured before. First, we validated the method by measuring the surface tension of water-in-oil microdrops deposited on a glass surface. We extracted an average tension of T~20.2 nN/μm, which agrees with macroscopic experimental methods. We then proceeded to measure cortical actin mechanical properties in non-adherent fibroblasts, and compare this to the properties after inducing two perturbations (i) adding blebbistatin which inhibits myosin II molecular motor activity, and (ii) adding CK-666 which inhibits Arp2/3-mediated actin branching. Our results show that perturbing the actin cortex had significant changes in each of the cortical mechanical properties: blebbistatin reduced them by ~50%, while CK-666 increased them by ~2-fold. These results validate our novel method for determining the quantitative mechanics of the actin cortex in eukaryotic cells.

**698-Pos Board B478**
**Relating Local Nanomechanical Response of Cells to Intracellular Forces and Cell Morphology**
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Mechanical processes regulate cell physiology primarily at the molecular level, but current techniques have difficulty achieving mechanical contrast at molecular length scales. We recently developed an AFM-based tool that can probe mechanical properties of living cells with nanoscale resolution. However, the measurements we obtained at the nanoscale are hard to reconcile with the viscoelastic view of cell mechanical behavior. We predominantly observe elastic response with little hysteresis in the corresponding force distance curves. In addition, force distance curves are surprisingly linear, which would not be the case for viscoelastic materials indented by conical AFM tips. We have created a model for the nanomechanical response of cells that takes intracellular forces into account. The model not only explains the near-elastic response and the linearity of force distance curves, but also makes quantitative predictions about cell shape and its relationship to the local nanomechanical response. We experimentally tested and verified these predictions on cells exhibiting different morphologies. In addition to these predictions, the model allows determining intracellular forces from the AFM images, such as tension across actin fibers and cortex tension. This work expands the existing cell mechanical models into the nanoscale and enables AFM to obtain physiologically relevant parameters from mechanical images.

**699-Pos Board B479**
**Co-Culture Changes the Mechanical Properties of Cells**
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Using an Atomic Force Microscope (AFM) with a 5.3 um diameter spherical probe we are measuring the elastic modulus of human mammary epithelial cells (HMEC) as they are co-cultured with immortal, tumorigenic, and finally metastatic. We are performing measurement over both normal cells and cancer cells. In order study the change induced by the co-culturing, our measurements will include the cell-pairs (normal cells and neighboring cancer cells).
So far, we found that normal cells show a significant difference in modulus after co-culturing with cancer cells. Measurements to date indicate that the moduli of HMEC increased more than 20% after co-culture with metastatic cells for 6 hours. We expect to report moduli under similar conditions for immortalized and tumorigenic HMEC cells and metastatic cells known as MDA-MB-231. In addition, we observe modulus differences due to transfection treatments. We also plan to report on these differences for immortalized, tumorigenic and metastatic versions of HMEC cells.
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**700-Pos Board B480**
**Quantitation of Compositional Changes in the Non-Erythroid Membrane Skeleton due to External Forces**
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The spectrin based membrane skeleton plays important roles in the mechanical and biological functions of living cells. Unlike the membrane skeleton of red blood cells, its non-erythroid counterpart has seen very little attention. Yet, it