Development of a Method for Quantitative Mechanical Nanotomography of Cells Embedded in 3D Matrixes

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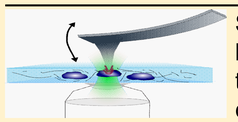
Research Objectives

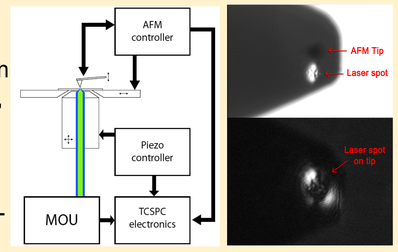


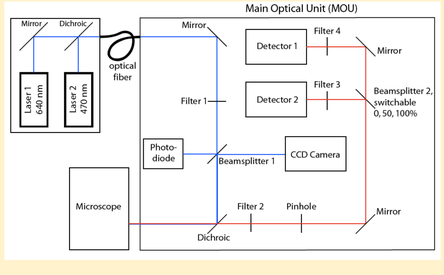
Several investigations of cancer cells have led to discoveries that cells of many cancer types are more elastic than their "normal" counterparts. The mechanical phenotypes of cells can tell us much about their physiology, behavior, and even their metastatic potential. But the mechanics of a cell can only be fully understood in the context of its microenvironment, the extracellular matrix (ECM), which is a complex meshwork of fibers and proteins which mediate the mechanical signals that cells send to one another. We use force spectroscopy and time-resolved fluorescence microscopy to probe the mechanical relationships between cells and the ECM in which they live. The specific aims of this project are:

- Development of mechanical nanotomography of cells embedded in true 3D matrixes for quantitative analysis of local mechanical properties.
- Development of gel encapsulation of living cells compatible with mechanical nanotomography. Cells' response to encapsulation in 3D matrixes of different stiffness and composition will be measured. This method will be applied to cells embedded in matrixes mimicking ECM.
- Development of a method based on combined AFM/optical microscopy to probe the response of the embedded cells to external mechanical stimuli.

Instrumentation

We use an Asylum MFP-3D BIO atomic force microscope (AFM) mounted on an inverted optical microscope, as depicted on the left [1]. Both the objective and sample positions are controlled with piezos. For combined measurements, the AFM tip is aligned with the confocal volume by using the laser light that is scattered back from the tip and captured by a CCD camera. Above, two views of the cantilever show the laser spot focused on and off the tip, respectively. Two pulsed lasers are used in an epifluorescence configuration. Emitted light is filtered by wavelength and sent to two detectors with single-photon sensitivity. The illumination pulses are time-tagged and the subsequently detected photons are time-correlated to extract the lifetime of the fluorophore's excited state. The AFM is used not only for scanning, but also for force spectroscopy measurements of the stiffness of cells with nanometer-scale spatial resolution and picometer-scale resolution in force. Below is a schematic of our optical setup.





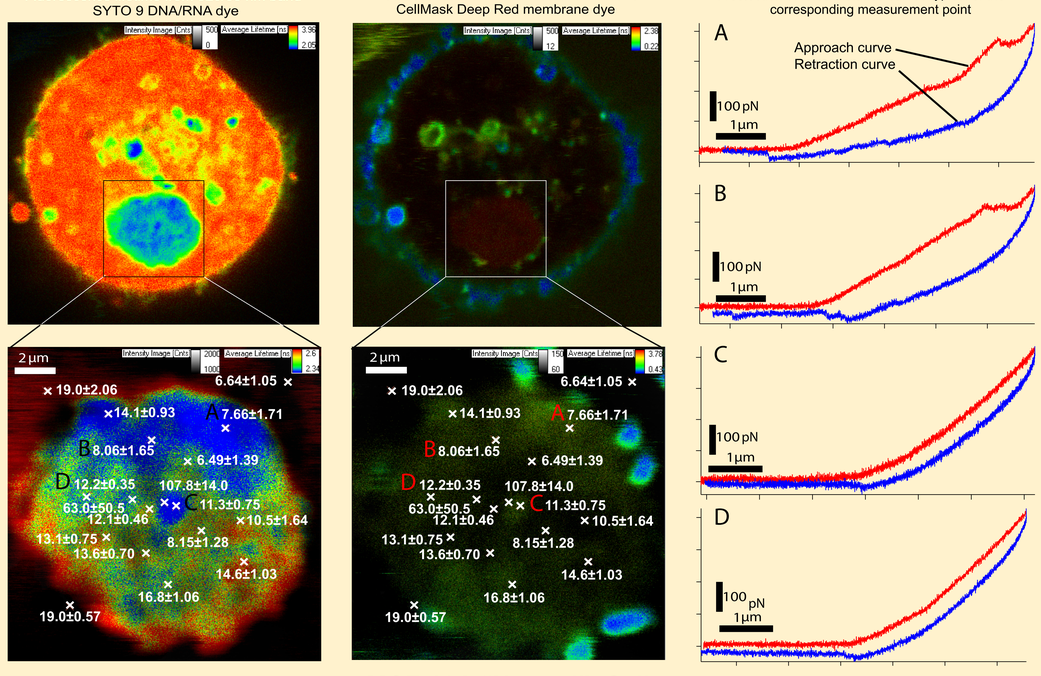
Young's Moduli of MDA-MB-231 Cancer Cells and MCF-10A "Normal" Cells Acquired by AFM Nanoindentation Correlated to Fluorescence Lifetimes

MDA-MB-231 Cancer Cell

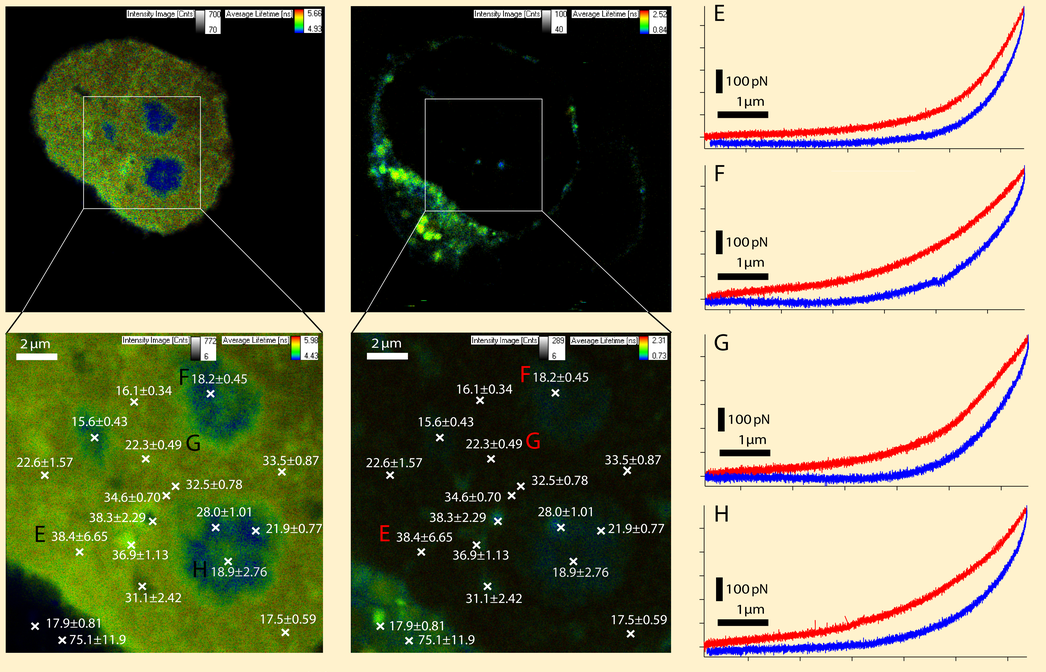
Fluorescence lifetime from 520/35 nm band SYTO 9 DNA/RNA dye

Fluorescence lifetime from 690/70 nm band CellMask Deep Red membrane dye

A selection of force-distance curves typical of the corresponding measurement point

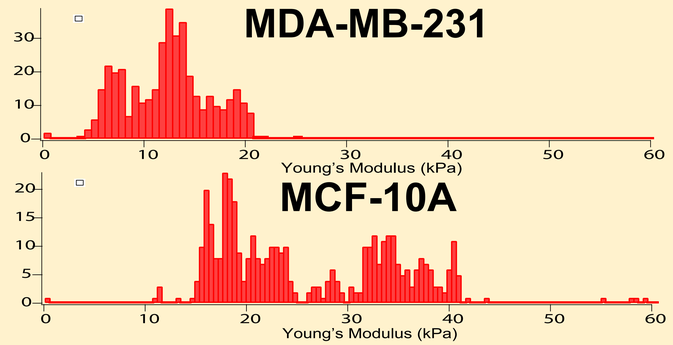


MCF-10A "Normal" Cell



Dual channel confocal images of the entirety and the nuclear region of single adherent cells from MDA-MB-231 and MCF-10A lines. At each point indicated by a white X, ~20 force-distance curves were recorded while that point was indented by an AFM tip. From each curve, a Young's modulus was calculated per the Hertz model. The Young's moduli mean ± standard deviation are indicated beside each indentation point. The large letters correspond to points from which representative curves are shown to

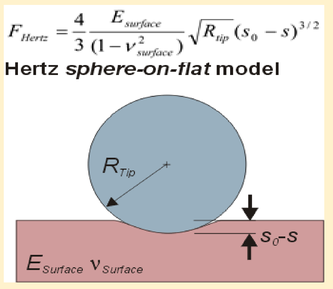
Single Cell Histograms of Young's Moduli of MDA-MB-231 and MCF-10A Cell Nuclei

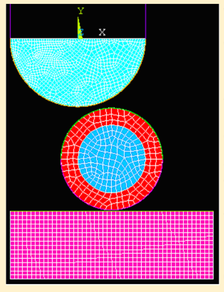


Young's moduli calculated from every force-distance curve collected from each of the two cells in the center panel are plotted in the histograms above.

The Future of AFM Cell Indentation

The standard models used to extract quantitative data from indentation experiments require homogeneous samples with uniform elasticity. Cells are extremely heterogeneous, and so we need more advanced models to understand cytomechanics.





With ANSYS finite element analysis software, we are developing models of AFM indentation experiments that incorporate the heterogeneity of the sample materials by varying the mechanical properties and geometry of their finite elements. These *in silico* experiments generate virtual force-distance curves that we compare to experimental data to better understand the composition of our samples.

Conclusions

Our single cell measurements confirm both that fluorescently labeled MDA-MB-231 cell nuclei are softer than those of MCF-10A cells, and that the distribution of Young's moduli throughout the nucleus is narrower in MDA-MB-231 than in MCF-10A cells. The observation of "scimitar" shaped force-distance curves (A and B), only present in MDA-MB-231, warrants further investigation. The shape of these curves is not correlated to Young's modulus. The nonlinearity of the "scimitar" curves demands the development of more sophisticated analysis tools.

References

[1] O. Schulz, F. Koberling, D. Walters, M. Koenig, J. Viani, R. Ros; Simultaneous single molecule atomic force and fluorescence lifetime imaging, Proc. of SPIE 7571, 757109 (2010).

Acknowledgments

This work was supported by the National Institutes of Health (U54CA143682), Arizona State University and GAANN P200A090123.