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Fluorescent Speckle Microrheology

MARGARET GARDEL, The Scripps Research Institute

The actin cortex is the dense shell of actin filaments between the cell membrane and the cytoplasm maintaining and regulating cell shape. It is one of the principal determinants of cell mechanical properties, whose spatiotemporal modulations play a central role in processes that involve architectural dynamics of a cell, such as cell migration, division and morphogenesis. However, the exact mechanism of cortical actin elasticity regulation *in vivo* is still unresolved. We present a high-resolution and molecularly specific assay of *in vivo* cortical actin elasticity, fluorescent speckle microrheology. Speckles originate when fluorescent actin is randomly incorporated into the network along with abundant endogeneous non-fluorescent actin, leading to high spatial variations of the local fluorophore density; high-density areas appear as diffraction-limited spots (speckles) upon high-resolution imaging. Speckles act as fiduciary marks of the network and can be used to directly image strain fluctuations, in contrast to classical microrheology techniques using imbedded probes. When tracking positional fluctuations of actin speckles in cells without convective network flow with subpixel precision, we find that the displacements of neighboring speckles are spatially correlated. Their correlation function decays as 1/r with interspeckle distance r, which is consistent with theoretical predictions for strain field decay in a 3D continuous viscoelastic medium. On the basis of these results, we use the amplitude of the correlation function to measure viscoelastic properties of the actin network. Due to high intracellular speckle densities and their homogeneous distribution throughout the cell, this approach yields much higher spatial resolution than other microrheology techniques, which is validated using *in vitro* actin networks. Thus, this assay allows us to map intracellular actin cortex elasticity with micron resolution, and to relate intracellular heterogeneities of elasticity to heterogeneities in other dynamic cellular parameters.