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**Fluorescent Speckle Microrheology of F-actin Networks** MARGARET GARDEL, M.I.T., Cambridge, MA USA, DINAH LOERKE, GAUDENZ DANUSER, CLARE WATERMAN-STORER, The Scripps Research Institute, La Jolla, CA USA — We present a non-invasive technique to probe the mechanical properties of F-actin cytoskeletal networks at sub-micron to micron length scales by using fluorescent speckle microscopy (FSM) to directly image the thermally-driven strain fluctuations of filaments in the network. In FSM, cytoskeletal polymers are labeled with a low concentration ratio of fluorescent:non-fluorescent cytoskeletal subunits and stochastic, spatial variations in fluorescence intensity result in diffraction-limited intensity peaks in high magnification, high resolution images called 'speckles'. Using TIRF microscopy and a fast, sensitive cooled CCD camera with on-chip multiplication gain, we were able to image speckles in *in vitro* F-actin networks cross-linked with $\alpha$-actinin at 30 frames/sec for nearly 120 seconds. We then track the thermally driven spatial trajectories of the speckles with subpixel accuracy and cross-correlate the displacements of pairs of speckles to directly map the strain fluctuations of the networks and use a generalized Stokes-Einstein relation to interpret these fluctuations in terms of the mechanical properties. Fluorescent speckle microrheology will be a powerful, highly spatiotemporally resolved method for non-invasively probing cytoskeletal mechanics in living cells during morphogenic processes such as migration or division.

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