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Fluorescence microscopy techniques for characterizing the microscale mechanical response of entangled actin networks SAVANNA BLAIR, TOBIAS FALZONE, RAE ROBERTSON-ANDERSON, University of San Diego — Actin filaments are semiflexible polymers that display complex viscoelastic properties when entangled in networks. In order to characterize the molecular-level physical and mechanical properties of entangled actin networks it is important to know the in-network length distribution and the response of entangled filaments to local forcing. Here we describe two single-molecule microscopy protocols developed to investigate these properties. Using confocal fluorescence microscopy and ImageJ image analysis we have developed a protocol to accurately measure the in-network actin length distribution. To characterize the deformation of actin filaments in response to perturbation, we trap micron size beads embedded in the network with optical tweezers and propagate the beads through the entangled filaments while simultaneously recording images of fluorescent-labeled filaments in the network. A sparse number of labeled filaments dispersed throughout the network allow us to visualize the movement of individual filaments during perturbation. Analysis of images taken during forcing is carried out using a combination of vector mapping and skeletonization techniques to directly reveal the deformation and subsequent relaxation modes induced in entangled actin filaments by microscale strains. We also determine the dependence of deformation modes on the relative filament position relative to the strain.

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