Characterization of Image Acquisition Noise in a Novel Fluorescence Speckle Microscopy Method

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Background: Dynamic changes in protein length (protein stretching events) have been observed in living cells using supra-resolution fluorescence microscopy. Our previous work documented the dynamic stretching of single molecules of talin, tagged with green fluorescent protein at the N-terminus and red fluorescent protein at the C-terminus (Margadant, et al., 2011). Previous speckle microscopy image analysis methods employed heuristics and assumptions that have not yet been analyzed for reliability.

Methods: A CUDA-accelerated simulator was implemented to emulate the 2D image acquisition process of talin labeled with red and green fluorescent proteins. The molecular lengths obeyed a user-defined probability density distribution. Each fluorescence tag was given an emission probability based on published literature. Localization error was added to emulate the diffraction limit of the instrument. Collisions, or signals of the same color located within a collision radius, were detected with two-pass counting.

Results: Four categories of assignments were studied in response to the following variable parameters: molecular density, collision radius, non-emitting tags, resolution limit, and molecular lengths. Jittering amplitude was found to have a profound impact on the observed histogram of molecular lengths.

Conclusions: Simulation of image acquisition was able to define the limit of observability, and to characterize the breakdown of molecular length interpretation subject to high density, low resolution, or low emission rate.

Keywords: speckle microscopy, image acquisition simulation, jittering, talin, protein stretching

References: