TIME-LAPSE IMAGING OF SINGLE-ANTIBODY LABELING ACHIEVES SUPERRESOLUTION

Hirushi Gunasekara[1], Thilini Perera[1], Jesse Anderson[2], Ying Hu[1].

[1]Department of Chemistry, College of Liberal Arts and Sciences; [2] Department of Chemical Engineering, College of Engineering; University of Illinois Chicago, Chicago, IL, 60607, USA.

Abstract:

We present a novel single-molecule localization microscopy technique that utilizes time-lapse imaging of single-antibody labeling to capture subcellular targets and generate superresolution images. By performing single-molecule imaging on a sub-minute timescale and optimizing the antibody concentration to create sparse single-molecule binding, we achieved high-resolution imaging of subcellular structures. Our observations indicate that local interaction densities in the cellular environment can effectively discriminate specific and non-specific antibody interactions. By gradually increasing the duration of the time-lapse between consecutive frames, we demonstrate enhanced capture of high-density interactions using monoclonal and polyclonal dye-conjugated antibodies alone or in combination. Using this approach, we demonstrate dual-target antibody labeling by combining a monoclonal anti-α-tubulin primary antibody and a polyclonal secondary antibody targeting the anti-Tom20 antibody. Moreover, we demonstrate a dual-color assay to improve the labeling density of the monoclonal hemagglutinin (HA) antibody and polyclonal $F(ab')_2$ antibody fragments. Single-antibody labeling provides a novel method to evaluate antibody binding for superresolution imaging in the native cellular environment.