Optimizing performance characteristics of antibodies for single-molecule quantitative bioimaging

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Antibody use is ubiquitous throughout biomedical research. Antibodies are applied to probe for proteins of interest, often in complex samples. Many of these techniques implicitly assume sufficient levels of antibody selectivity and specificity. Single-molecule fluorescence microscopy (SMFM) is a highly sensitive method of visualizing proteins in situ, capable of detecting single antibodies in high-background samples. SMFM is especially vulnerable to false positives due to its unparalleled sensitivity: any off-target binding event will produce a false positive signal. Recently, we employed SMFM to quantify disease-relevant alpha-synuclein oligomers in human brain tissue. Especially in this size domain (< 250 nm) antibodies with low selectivity produce many biological false-positives due SMFM's sensitivity. Here, we present a novel, high-resolution quantitative fluorescence microscopy bioimaging pipeline. This method quantifies and optimizes the selectivity and specificity of antibodies for use in SMFM, with rapid acquisition, processing and quantification of (false) positive antibody binding events. We identify thousands of oligomers of alpha-synuclein (< 250 nm) in mouse brain. Here, we find significant levels of off-target interactions of multiple commercially available alpha-synuclein antibodies with other proteins which yield false positive signal in SMFM. We are able to reliably quantify antibody selectivity and sensitivity, specifically for the application of SMFM. Our findings showcase a pipeline for the optimization of antibody selectivity and specificity for the purpose of SMFM which will be beneficial for the quickly evolving field of high-resolution microscopy with applications such as super-resolution microscopy techniques, SiMPull and, more generally, any technique reliant on antibodies.