Proteins will often dynamically transition between complexes of varying number of subunits depending on the inter- and intracellular environment. Labeling these subunits with fluorophores allows them to be localized to high precision with several super-resolution microscopy techniques, but quantifying the number of subunits in a sub-diffraction-limited area is complicated by the photophysical properties of the fluorophores. Blinking, re-activation, and complex kinetics all make identifying single molecules difficult.

Here we present a technique to utilize these fluorophore photophysics to quantify cluster numbers in a serial localization microscopy domain. Unlike previous techniques, this approach utilizes spatial and temporal information to form adaptive criteria for differentiating single fluorophores from higher clusters, avoiding both over- and undercounting errors in the quantification process. This method can correctly identify the number and nature of molecular clusters with 98% accuracy on simulated data.