**Text S6. Building Trajectories from Localizations.**

Cost matrices are used to build trajectories in two steps: frame-to-frame and gap closing. Physical models are used to derive the costs for linking localizations, starting a new trajectory (birth), and ending a trajectory (death). The cost for connecting observations 1 and 2 is

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Under the assumption of Brownian motion, the probability of making observation 2 () given observation 1 () is

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where

is the spatial localization error for observation 1 and is similarly defined for observation 2. Similarly, due to the spectral emission peak, the probability of making observation 2 ( given observation 1 ( is

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where accounts for the variance in the observed spectral emission peak due to spectral jumping or bluing of individual QDs. The cost for a previously unobserved QD blinking on (birth) or a currently tracked QD blinking off (death) is determined by particle density and estimated blinking rates. Building a cost matrix from physical models is discussed in more detail in a manuscript in preparation by Relich P, Cutler PJ, Huang F, Lidke KA.

The cost matrix built from costs of linking, birth, and death is treated as a linear assignment problem to link localizations into trajectories. The spectral information of localized QDs greatly improves the accuracy of trajectories and permits SPT at higher labeling densities (**Figure S14**).