**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

$l\_{m,n}=-log⁡\left(P\left(x\_{1},y\_{1},t\_{1}\right)P\left(λ\_{1}\right)\right)$.

Under the assumption of Brownian motion, the probability of making observation 2 ($x\_{2},y\_{2},t\_{2}$) given observation 1 ($x\_{2},y\_{2},t\_{2}$) is

$P\left(x\_{1},y\_{1},t\_{1}\right)=e^{\frac{-\left(θ\_{x\_{1}}-θ\_{x\_{2}}\right)^{2}-\left(θ\_{y\_{1}}-θ\_{y\_{2}}\right)^{2}}{4DΔt+2σ\_{1}^{2}+2σ\_{2}^{2}}}$,

where

$$σ\_{1}^{2}=σ\_{θ\_{x\_{1}}}^{2}+σ\_{θ\_{y\_{1}}}^{2}$$

is the spatial localization error for observation 1 and $σ\_{2}^{2}$ is similarly defined for observation 2. Similarly, due to the spectral emission peak, the probability of making observation 2 ($λ\_{2})$ given observation 1 ($λ\_{1})$ is

$P\left(λ\_{1}\right)=erf⁡\left(\frac{\left|θ\_{λ\_{1}}-θ\_{λ\_{2}}\right|}{\sqrt{2\left(σ\_{θ\_{λ\_{1}}}^{2}+σ\_{θ\_{λ\_{2}}}^{2}+σ\_{λ\_{jump}}^{2}\right)}}\right)$,

where $σ\_{λ\_{jump}}^{2}$ 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**).