Multi-frame particle detection that enables robust single molecule tracking.

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KEY WORDS: Single molecule invivo imaging and detection, low light detection, Photosensitivity, Hypothesis testing, mRNA, nucleocytoplasmic transport

In the cell mRNAs are known to export from the nucleus to the cytoplasm. To visualize the dynamics of fluorescently labeled mRNA using MS2 stem loops and single molecule tracking, millisecond temporal resolution (Δt = 20 ms) is essential as mRNA shows diffusion in the 1-5 µm²/s range. However, the dynamics of mRNA mobility critically depend on continuous tracking of mRNAs as trace interruptions will lead to an overestimation of mobility. This has been a challenge to automated image analysis making it the bottleneck of single-molecule experiments in the living cell.

The consequence of these fast acquisition frame rates in single molecule in vivo imaging is that only a few photons can be collected from each molecule in each frame (I=1-25 #). Imaging in the nucleus is further complicated by a high background signal (b=90 #). This results in a low SNR (I/sqrt(I+b)=0.1-2.3), which compromises the localization precision. We therefore use detection theory to estimate the location of single molecules and to validate this estimation.

The theoretical approach that we use is a hypothesis test between a) the absence of a single emitter (H₀) and b) the presence of a single emitter (H₁). This approach is further optimized by taking into account the whole time series recorded.