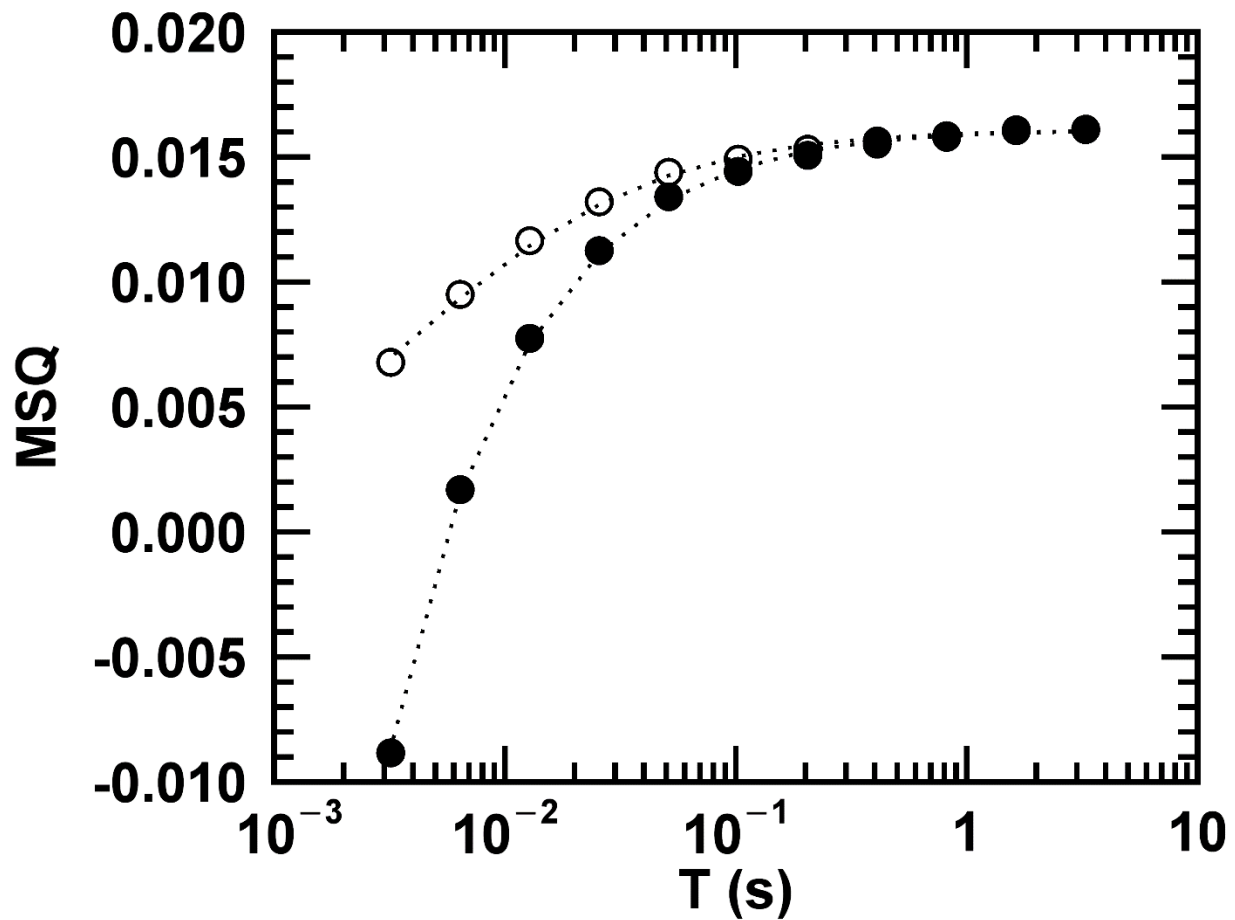# Supplementary Materials

Supplementary Fig. S1. MSQ curves with and without the shot noise term. The MSQ curves from an FFS experiment conducted in the cytoplasm of cells expressing EGFP before (filled circles) and after (open circles) shot-noise subtraction differ at short segment times T. The shot noise term, which was included in the original formulation of MSQ, increases in magnitude as the segment time decreases and starts to dominate the MSQ amplitude.
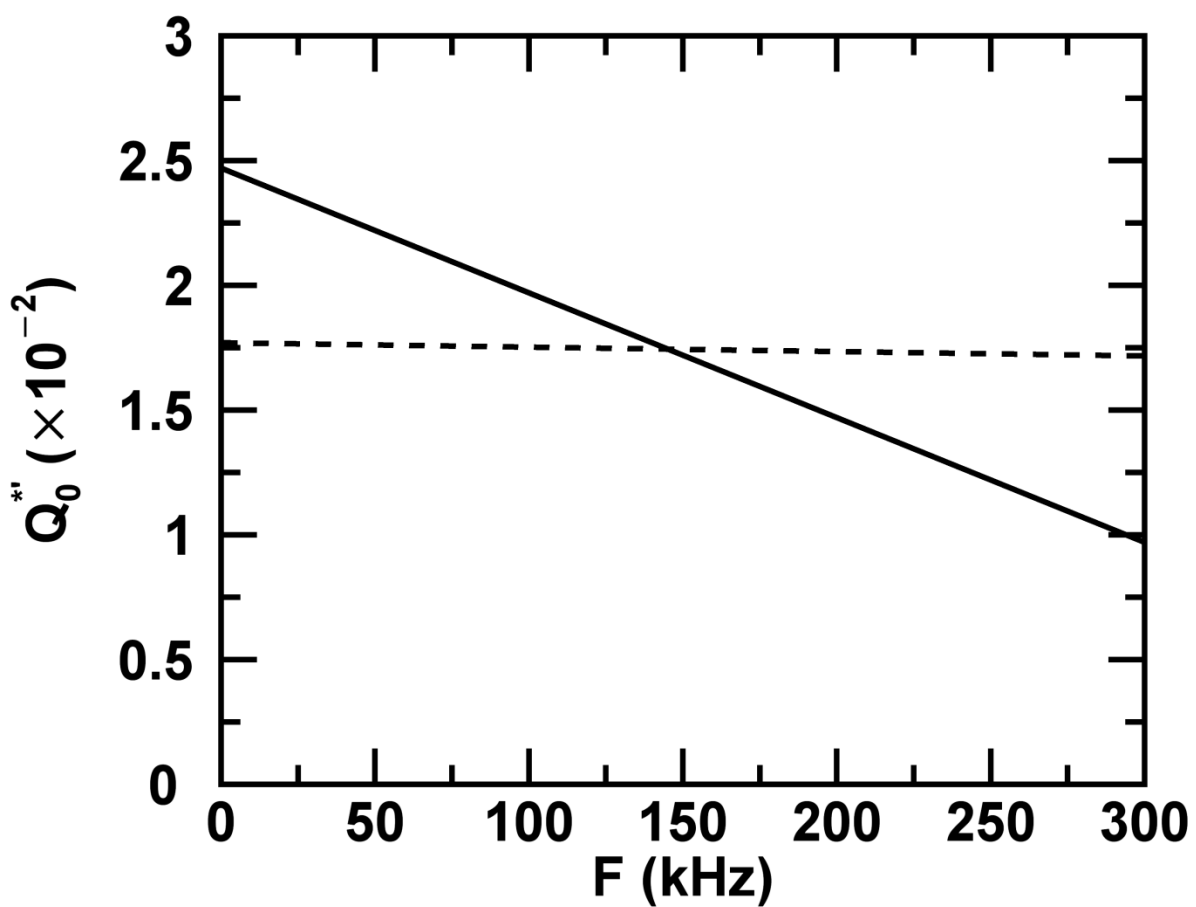
Supplementary Fig. S2. The effect of dead-time and afterpulsing on the MSQ (solid line) and tsMSQ (dashed line) amplitude $Q_0^{*\prime}$ **as a function of fluorescence intensity.** The biased $Q_0^{*\prime}$ value was modeled for an afterpulsing probability of P = 0.0035, a dead-time of $\tau_\dagger$ = 25 ns, a sampling time of 50 µs, and an ideal Q_0 of 0.0177.
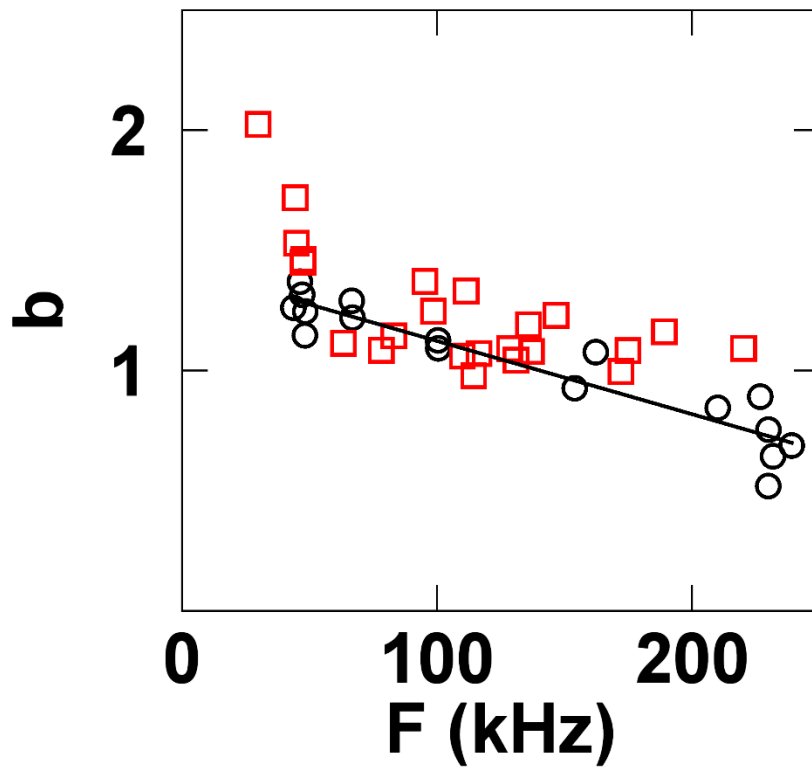
Supplementary Fig. S3. Comparison of MSQ results for EGFP in the cytoplasm and NE . Biased brightness from MSQ vs. intensity for EGFP in the cytoplasm (black circles) and linear fit (solid line) was taken from Figure 3B for comparison with the biased brightness for SS-EGFP in the NE (red squares) from Figure 3C.