

Fig. S1 (a) Parametric Convergence for 450nm-excited Monte Carlo Simulations (b) Parametric Convergence for 680nm-excited Monte Carlo Simulations

Supplementary Note S1. Explanation of Parametric Analysis

Supplementary Fig. S1 shows parametric convergence for two biosensor iterations: one with an excitation of 450nm and one with an excitation of 680nm. As described in section 2.6, excitation and emission simulations with a target fluorophore of absorption coefficient and power yield of 0.001cm⁻¹ and 0.001 respectively were carried out with increasingly many photons in triplicate. It was found that the fluorescence intensity reaching the detector when 10⁷ photons were used was within 5% of the fluorescence intensity reaching the detector when 10⁸ photons were used, indicating that simulation output has converged at 10⁷ photons in both cases.

Supplementary Note S2. Number of Simulations Required for a Single Barcode Biosensor Iteration

Phosphorescence Lifetime Assay:

- 1. 1 excitation simulation (x3 for triplicate. 3 total)
- 2. 80 emission simulations (x3 for triplicate, 240 total)

FRET Assay

- 1. 1 excitation simulation (x3 for triplicate, 3 total)
- 2. 80 donor dye emission simulations (x3 for triplicate, 240 total)
- 3. 80 acceptor dye emission simulations (x3 for triplicate, 240 total)