

Time (s)

# of donor and acceptor photons

Fluorescence photon count time trace

compute  $E1$  and  $E2$

if  $\Delta t < \text{time window}$   
add point to  $(E1, E2)$  bin

2D FRET efficiency histogram

$E2$

$E1$

$(E1, E2)$  bin

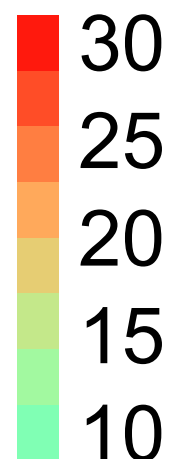


Figure 2 - figure supplement 3