Supplementary Method

Contribution from cross-excitation of RFP can be removed in the following steps:

1) Use an RFP construct to obtain the correction factor, defined by YR/RR, where YR and RR represent readings in the RFP intensity upon excitation of YFP and RFP, respectively;

2) Measure the direct RFP emission to obtain total RFP (RR') in cells where correction is required; and

3) Multiply the correction factor (YR/RR) with the total RFP direct emission (RR') to determine the contribution of cross-excited RFP upon YFP excitation (YR_x), which will then be subtracted from the experimental reading in the YR-FRET channel (YR_{exp}).

   Thus, after correction, the actual YR-FRET signal (YR_{actual}) can be calculated as

   $$YR_{actual} = YR_{exp} - YR_x, \text{ where } YR_x = RR' \times (YR/RR).$$