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Electronic Supplementary Materials

Below is the link to the electronic supplementary material.

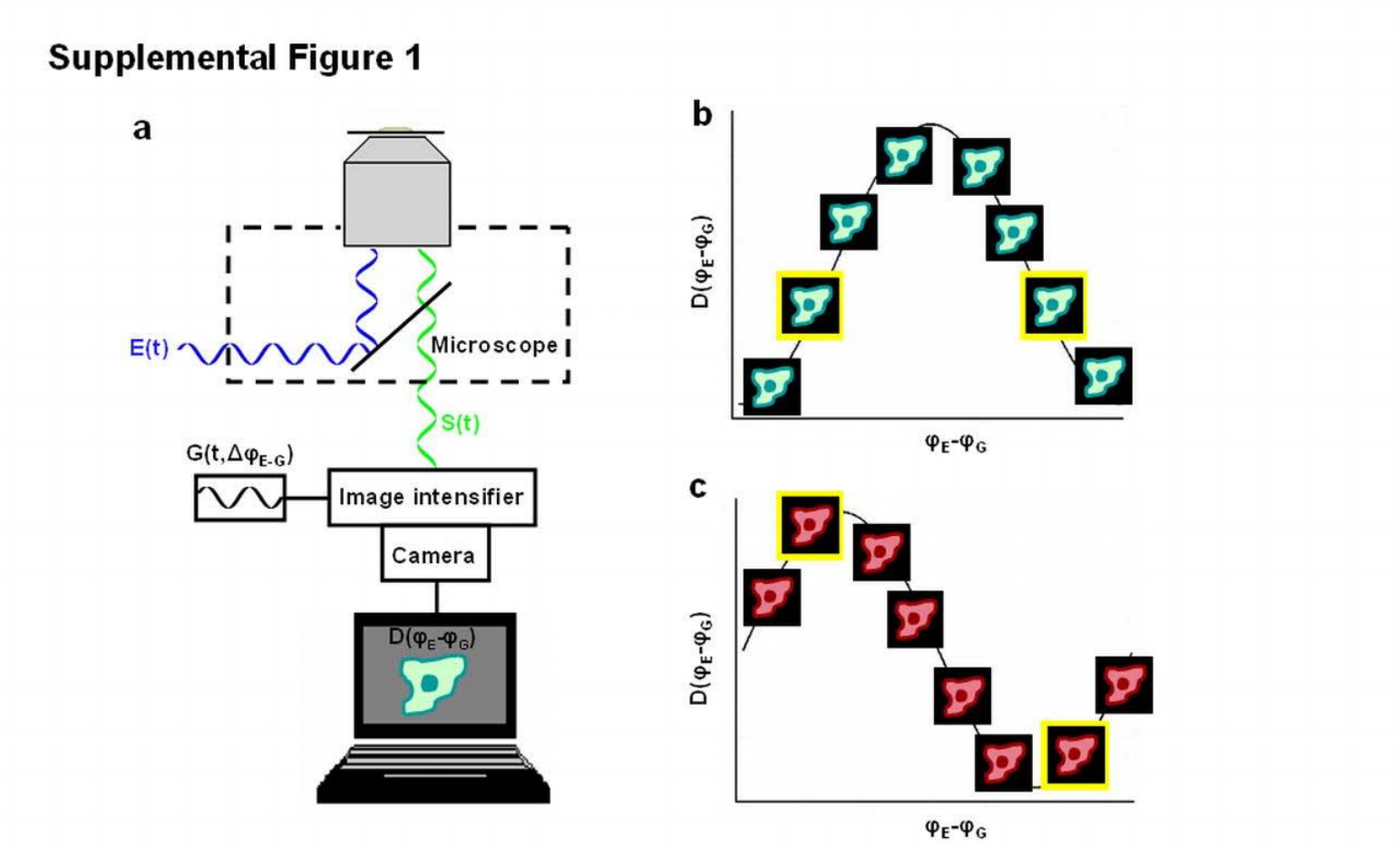

Fig. S1 Full-field FLIM setup and detection: (A) The excitation light *E(t)*, of the full-field FLIM instrument has its intensity modulated prior to being incident on the sample. The sample's fluorescence emission described as *S(t)*, will also have its intensity modulated at the same frequency as the exciting light. As a result of the interaction with the sample, the modulated emission will be phase shifted and de-modulated relative to the excitation light. In the detector system, a separate sinusoidal signal *G(t)* is injected and mixed with *S(t)* (sample's emission) to simplify the determination of lifetimes by homodyning. (B) An example of the eight images measured at the different phases of *G(t)* over a full period are shown. It is the modulation depth (similar to amplitude) and the phase of this curve both relative to that of a known standard, that can be used to extract lifetimes. (C) A separate set of images collected at the same set of phases of *G(t)* as in (B) are phase shifted with respect to those in (B). The phase shift relative to the curve described in (B) is indicative of a different lifetime. Highlighted in yellow is an example of images that could be used for phase differential enhancement or possibly phase suppression. (JPEG 67.2 kb)

High resolution image (TIFF 104 kb)

Supplemental Figure 2

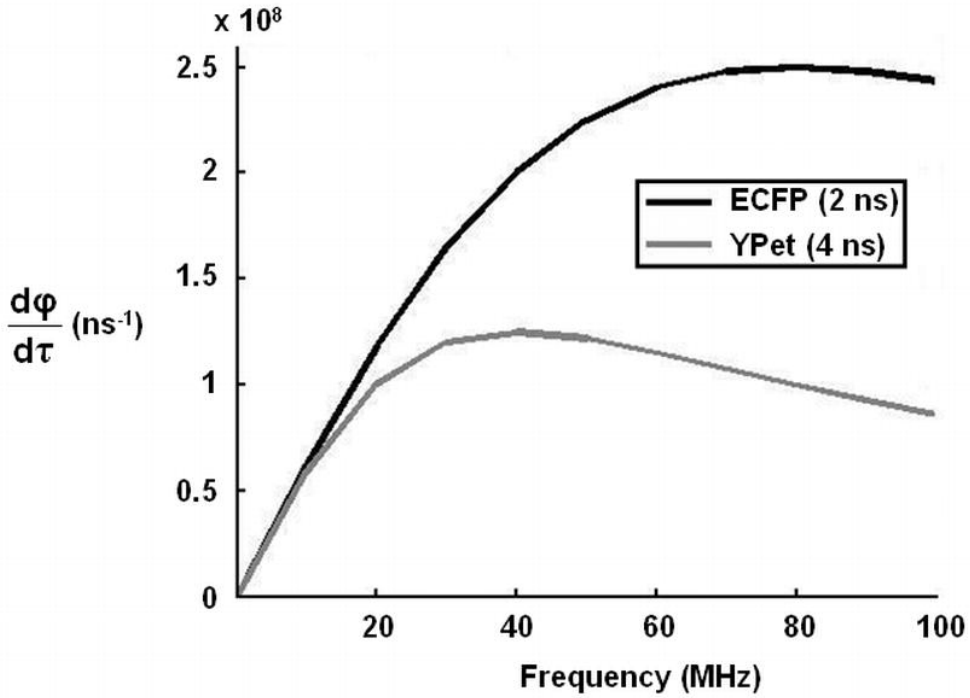

Fig. S2 Optimizing the modulation frequency for the detection of the biosensor's conformation with phase suppression or phase differential enhancement: In order to get the greatest change in phase delay for small changes in lifetime in a specific range of lifetimes, the choice of the modulation frequency can be performed with optimization. The optimum modulation frequency necessary to provide large changes in phase delay for an approximate range of lifetimes can be determined by taking the derivative of the phase delay with respect to the lifetime. As a function of frequency with a given lifetime (or lifetime range), this derivative will have a peak at the frequency where there is the highest change in phase delay per small change in lifetime. Hence, it is the frequency at this peak that will provide the greatest separation in intensity between species near the given lifetime when applying phase differential enhancement or phase suppression. (JPEG 42 kb)

High resolution image (TIFF 75.7 kb)

Supplemental Figure 3

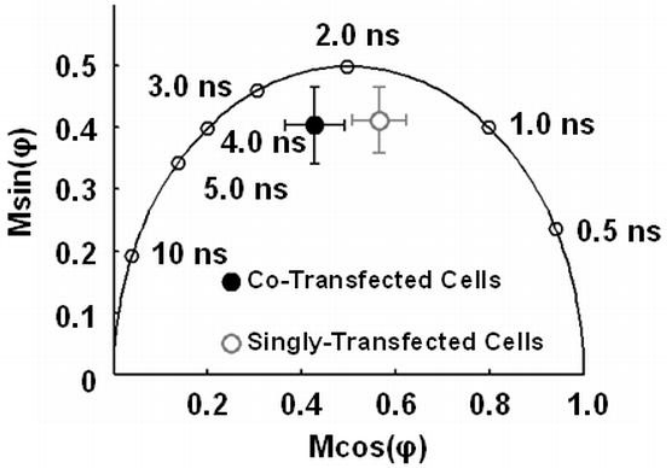

Fig. S3 Singly and co-transfected HeLa cells imaged through the ECFP channel at 80 MHz: This polar plot describes the HeLa cells examined for the phase suppression analysis. The polar coordinate shown in red represents the HeLa cells singly transfected with the MT1-MMP biosensor. When the HeLa cells were co-transfected with the MT1-MMP biosensor and the MT1-MMP enzyme, the polar coordinate (shown in green) is shifted toward the region of longer lifetimes indicating a reduction in FRET. The error bars indicate standard deviation along x and y. (JPEG 42.3 kb)

High resolution image (TIFF 60.7 kb)

Footnotes

1 Personal Communication