Supplementary

Table 1. Comparisons of several common fluorescence-based imaging approaches that can be used for the visualization of cell-substrate interactions.

Microscopy tool	**Pros**	**Cons**
Total internal reflection fluorescence microscopy	Excites fluorophores near the adherent cell surface and minimizes fluorescence originating from the bulk of the cell	Florescence intensity is dependent upon both the local dye concentration and the position of the emitters with respect to the substrate surface
Fluorescence confocal microscopy	Generates high-resolution image in three dimensions	Background excitation of components that belong to the cell body Low throughput
Surface plasmon enhanced fluorescence	Amplifies excitation light, alter the spatial distribution of the fluorophore emission and modify the radiative lifetime of the fluorophore	Strong fluorescence quenching for fluorophores in close proximity to metal Low quality factor resonances

Figure 1. Plot of the measured average fluorescence intensity for five sequential scans under off-resonance illumination with plasma membrane dye (CellMask™ Deep Red Plasma membrane Stain) and nucleus dye (NucRed™ Live 647 ReadyProbes™ Reagent). The fluorescence intensity is normalized to the value of initial measurement. The fluorophores in subsequent scans are 64%, 46%, 30% and 21% of the initial value for the plasma membrane dye and 92%, 89%, 88% and 86% of the first scan for the nucleus dye.