HEK293T cell imaging protocol
(This protocol can be used for cell imaging in other common mammalian cell lines)

1. Coat glass-bottom dishes (Mattek #P24G-1.5-13-F) with 100 μg/mL of poly-L-lysine (PLL) overnight at 4°C.
2. Wash the dishes twice with ddH_2O and UV sterilized for 5 min. Coat the sterilized dishes with 100 μg/mL rat collagen-I and 50 μg/mL laminin for 2 hours at 37°C.
3. After removing the coating solution and allowing the dishes to air-dry, plate HEK293T cells at 80,000 cells/ml.
4. After 24 hours, transfect HEK293T cells with 0.4 μg of Spinach2-tagged RNA construct with FuGene HD [0.4 μg DNA, 1.8 μL FuGene HD (Promega #E2311), in Opti-MEM => 20 μL/well]. Imaging experiments are typically performed 48 hours after transfection.
5. Thirty minutes prior to experiment, replace HEK293T cell media with imaging media (DMEM with no phenol red or vitamins and supplemented with 25 mM HEPES, 5 mM $MgSO_4$, and 5 μg/ml Hoechst 33342) and 20 μM DFHBI-1T or vehicle are added to the cells to promote RNA-fluorophore complex formation.
6. Live fluorescence images of HEK293T cells are taken with the DIC (Normal) microscope using the 100X oil objective.
7. DFHBI-1T fluorescence are detected using the FITC filter (~100 ms exposure), Hoescht 33342 fluorescence with the DAPI filter (~100 ms exposure,) and phase-contract images are taken at ~500 ms.
8. To remove any green autofluorescence or residual fluorescence from DFHBI-1T, take images of untransfected control cells with DFHBI-1T and subtract this background fluorescence from Spinach2™-containing cells.

Note: Unless specifically stated, all reagents were purchased from Life Technologies.