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A Morphological Identification Cell Cytotoxicity Assay Using Cytoplasm-localized Fluorescent Probe (CLFP) to Distinguish Living and Dead Cells

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CLFP staining

The fluorescence microscopy was performed with high content screening system (Operetta) PerkinElmer and/or fluorescence microscopy Olympus IX71.

1 Staining conditions for CLFP probe 1 and 2

HepG2 cells were cultured in 24-well plates with low density. Two CLFPs 1 and 2 were reported previously (Figure S1), the experiment condition of CLFP 1 is 25 μM × 30 min, UV excited; and probe 2 is 1 μM × 10 min, blue fluorescence excited. After incubation, the medium was washed away with PBS buffer, HepG2 cells were imaged under fluorescent microscopy. Both CLFP 1 and 2 staining can provide reliable and high resolution cell images (Figure S2)

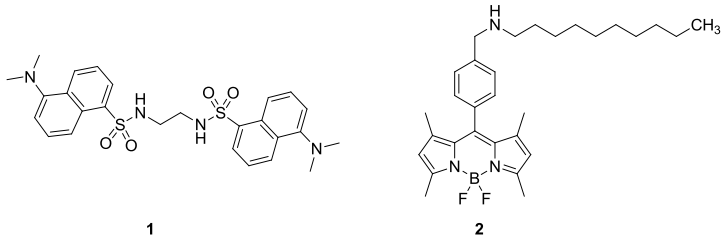

Figure S1. Structures of two CLFPs reported in our previous study.26 Probe 1 is a DNS-derived CLFP, and probe 2 is a BODIPY-derived CLFP.

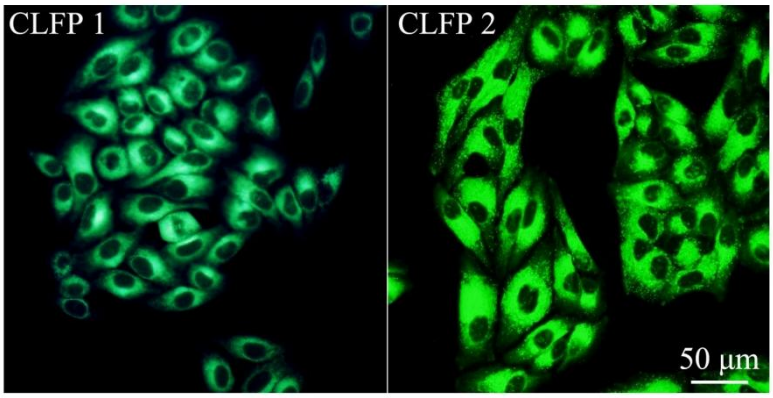

Figure S2. Fluorescence microscopy images of the morphologies of HepG2 cells, which show the cytoplasm localization of probe 1 and 2. Both probe staining can give good quality cell images.

2 The imaging of fluorescent morphologies of dead cells using probe 2 staining

HepG2 cells were cultured into 24-well plates and treated with various concentrations of cytotoxicity drugs (Paclitaxel, Staurosporine) for 16 hours. The concentrations of these compounds were varied among 0.001 μM to 0.1 μM. After washed with PBS for 3 times, probe 2 was added into the wells for an hour to stain HepG2 cells, and imaged under fluorescent microscopy.

As long as the HepG2 cells were treated under the same conditions, CLFP 2 staining provides similar fluorescent images as CLFP1 staining. Figure S2 show the morphologies of dying cells as bright, solid fluorescent spheres (white arrows). The intensity of these fluorescent spheres was stronger than that of living cells. This enhanced the pan-applicability of CLFP method.

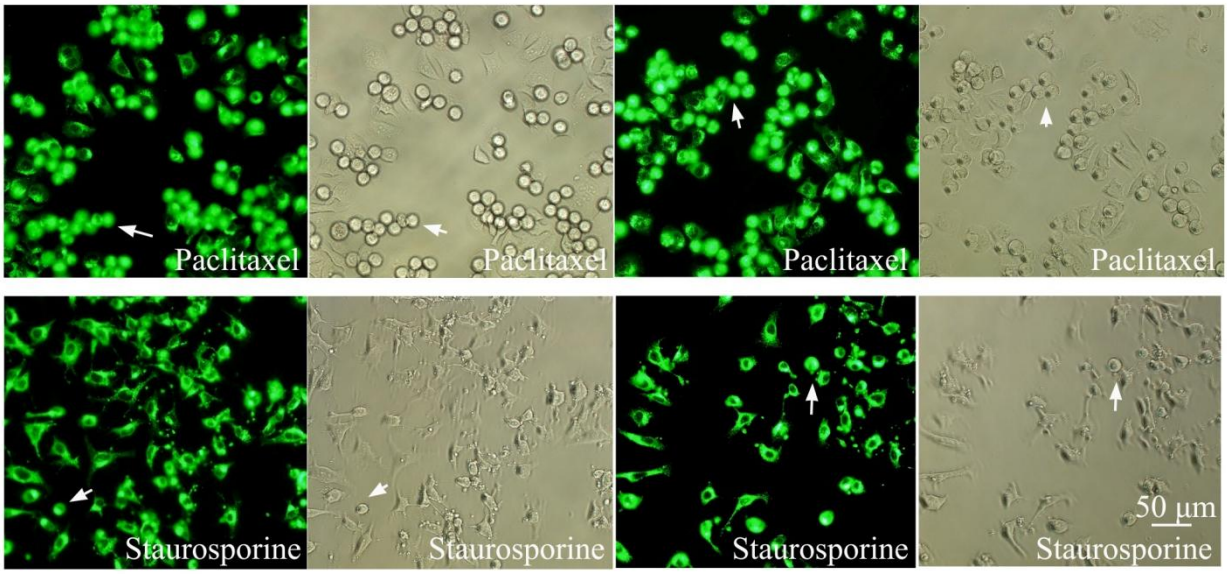

Figure S3. HepG2 cells images, which were treated with various concentrations of cytotoxicity drugs (Paclitaxel, Staurosporine). HepG2 cells then were stained with probe 2, and imaged under both fluorescence and optical microscopy. For all of the dead cells that can be observed in the optical with round shapes, we can see that they show enhanced fluorescent intensities and full shape of fluorescence in the fluorescent images. This is consistent with the CLFP 1 staining.

3. Series of paclitaxel-treated HepG2 cells were stained with CLFP 2

HepG2 cells were treated with different concentrations Paclitaxel (0, 0.0016, 0.008, 0.04, 0.2, 1μM) for 24 hours. Cells were then stained with probe 1 for 10 min. After washing, the cells were imaged under fluorescent microscopy. Figure S4A–F show the ratio of healthy cells to dead cells in different concentrations of

paclitaxel-treated HepG2 cells. As the concentration of paclitaxel increased, fewer healthy cells were observed, whereas the number of dead cells initially increased to a maximum (Figure S4D), and then decreased (Figures S4E and S4F). This is also in agree with the CLFP 1 staining.

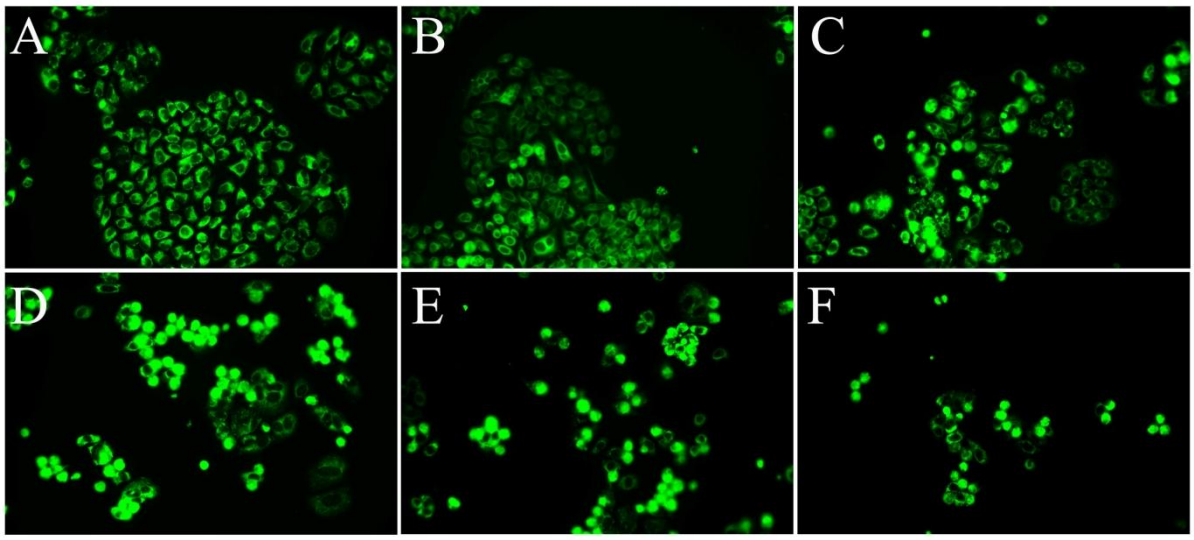

Figure S4. (A–F) Fluorescence microscopy images of the morphologies of HepG2 cells treated with a series of concentrations of paclitaxel.

4. Computation analysis of the fluorescent photos.

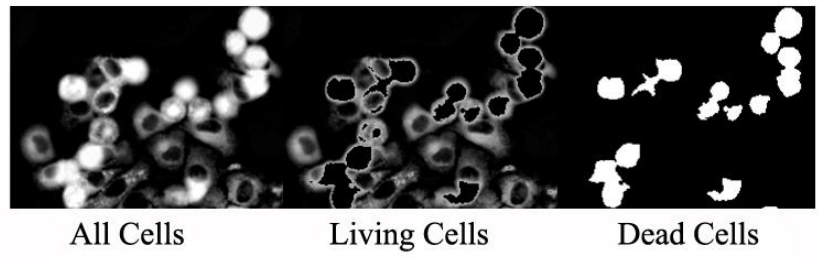

Figure S5. Anatomy of the computational analysis used to separate the areas of living and dead cells from the grayscale fluorescence microscope image.