Cell imaging with squaraine dye based on two-photon excitation fluorescence imaging

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Extended abstract

Cancer is one of the major killers in most areas of the world. Due to its high mortality rate, more and more research team have devoted themselves to diagnosis and treatment approaches. Because of its advantages of low cytotoxicity, high resolution and strong penetration, two-photon excitation fluorescence imaging (TPEFI) has become one of the most promising imaging modality for cancer diagnosis. Compared with the traditional single-photon imaging technology, it has many advantages such as little light damage, small bleaching area, high spatial resolution, high fluorescence collection efficiency, and high image contrast. Especially, two-photon microscopy is more suitable for the observation of thick specimen, and real-time three-dimensional or four-dimensional observations of living cells and even living tissue. However, some probes used to label living cells are toxic and will have an adverse effect on the living cells imaging. Squaraine dye is an organic dye with strong fluorescence and low cytotoxicity. Therefore, the purpose of this study is to investigate the imaging of living cells based on two-photon imaging using squaraine dye.

In this study, we cultured OVCAR-3 ovarian cells for our imaging, and also synthesized a squaraine dye which had the significant fluorescent effect. Firstly, the single-photon emission spectrum of the squaraine dye was measured and the result is shown in figure 1(a).

Then we measured the two-photon emission spectrum of the squaraine dye (figure 1(b)).

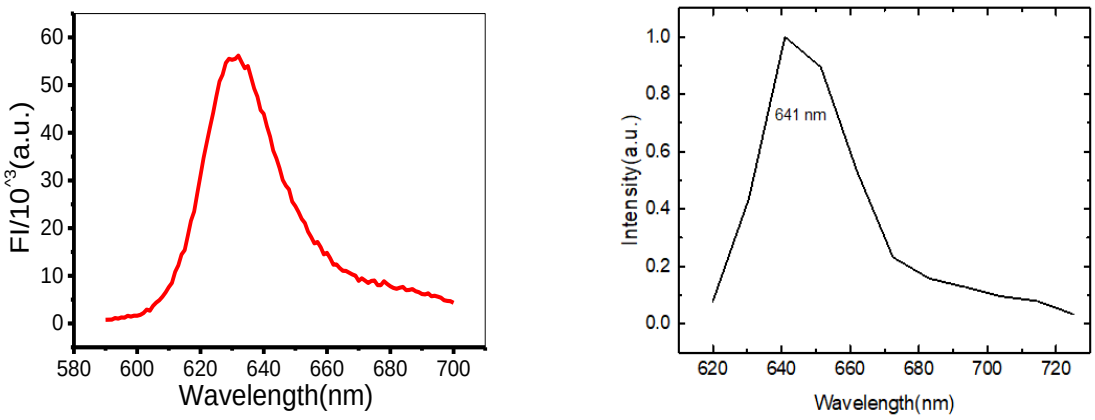

Figure 1(a). Single-photon emission spectrum of squaraine dye. (b). Two-photon emission spectrum of squaraine dye in OVCAR-3 cells

Based on the spectrum, we selected 600 nm as single-photon excitation wavelength, and performed single-photon imaging using Leica sp8 (figure 2(a)). Using 1000 nm as emission wavelength, two-photon imaging was also carried out, and the image is shown in figure 2(b).

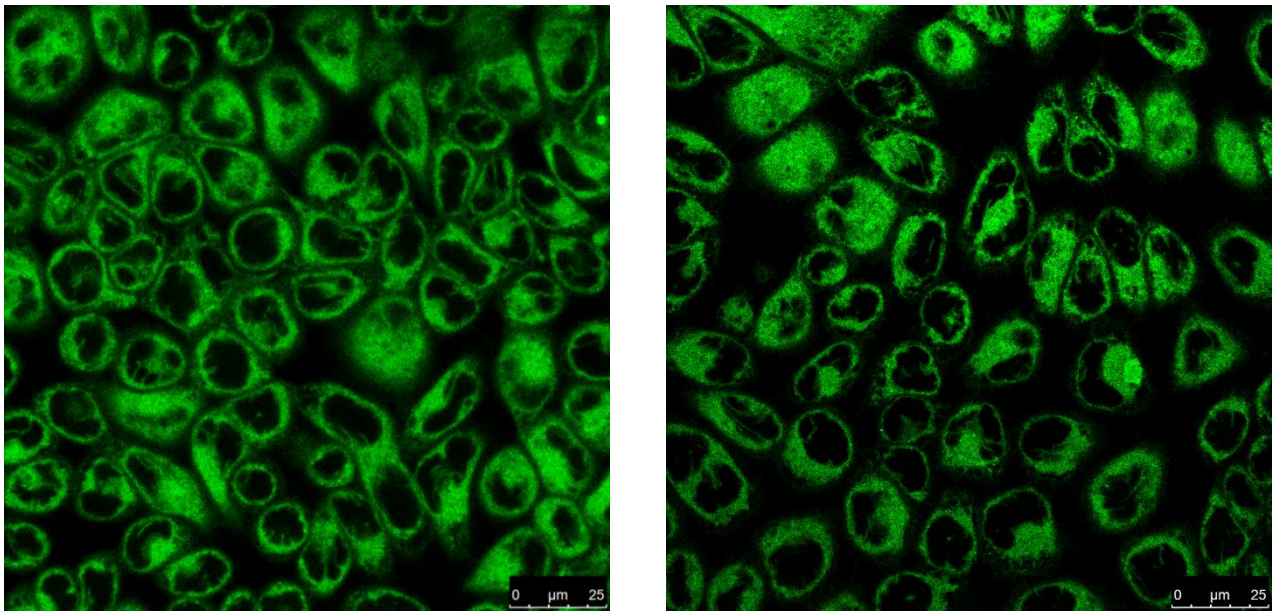

Figure 2(a). Single-photon imaging of OVCAR-3 cells. The cells were co-incubated with 1 µ mol L^{-1} squaraine dye for 0.5 h and washed with PBS 7.4 three times.Figure 2(b). Two photon cell imaging of OVCAR-3 cells. The cells were co-incubated with 1 µmol L^{-1} squaraine dye for 0.5 h and washed with PBS 7.4 three times.

Compared with the one-photon image, the two-photon image had higher resolution and deeper imaging depth. In conclusion, two-photon imaging using squaraine dye is a promising imaging technique that may be widely applied in cancer diagnosis in the future.