Electronic Supplementary Material (ESI) for RSC Advances.

Table S1 Viscosity of water/glycerol system

water/glycerol（v:v）	η(cP)
100:0	1.03
50:50	5.42
40:60	10.41
30:70	27.81
20:80	57.61
15:85	126.05
10:90	177.63
8:92	337.50
6:94	458.43
2:98	721.28
0:100	952.88

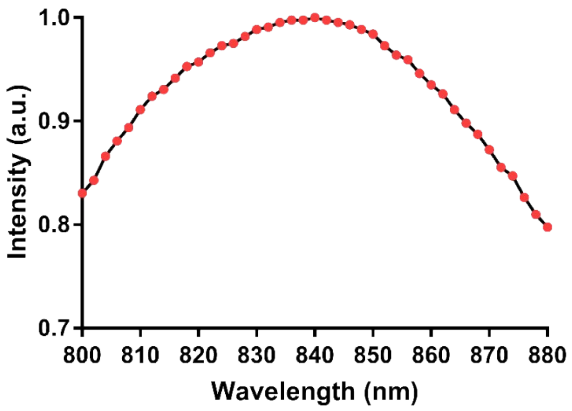

Fig. S1. The two-photon absorptivity of femtosecond laser with different central wavelengths of TPA-Mit. The excitation efficiency of TPA-Mit researched to the highest when the excitation wavelength was 840 nm.

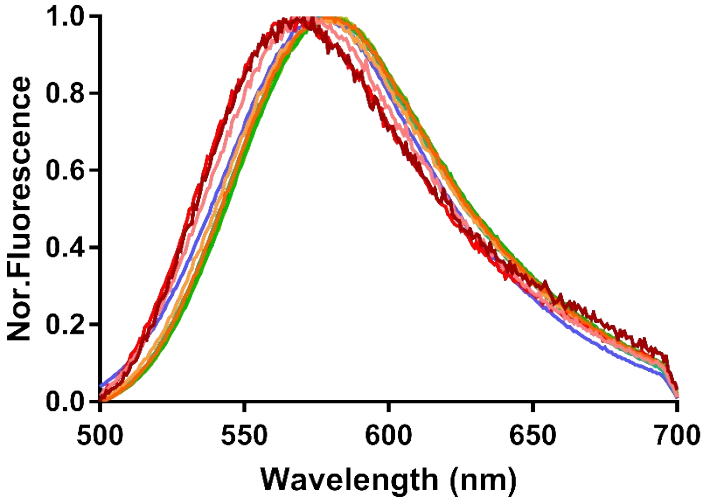

Fig. S2. Fluorescence emission normalised spectra of TPA-Mit under different solution viscosities in the water/glycerol system (λex =840 nm, λem = 580 nm).

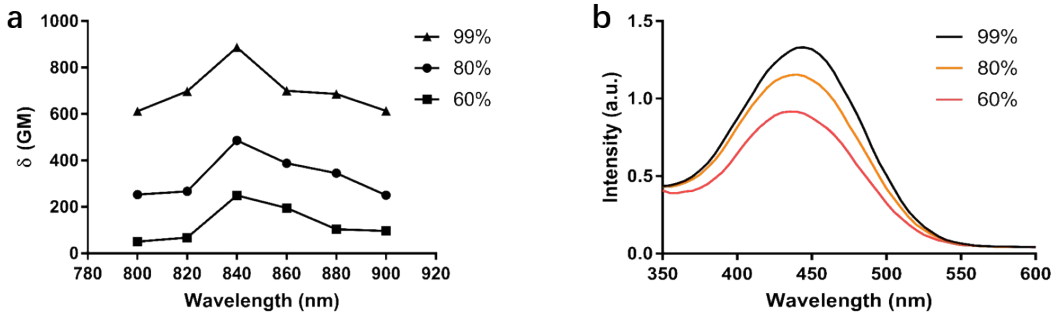

Fig. S3. (a) Two-photon absorption cross-section of TPA-Mit under different solution viscosities in the water/glycerol system. (b) Absorption spectra of TPA-Mit under different viscosities

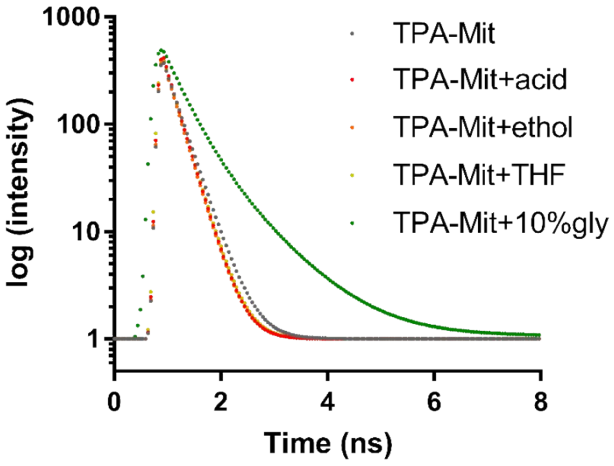

Fig. S4. Fluorescence lifetime of TPA-Mit in different solution environments.

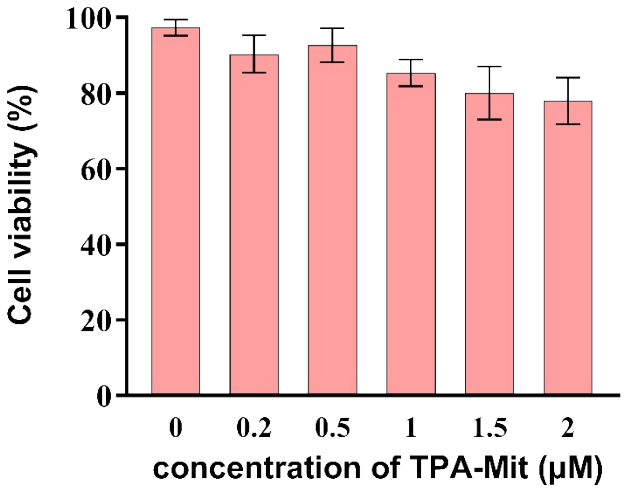

Fig. S5. Viability of SKOV-3 cells after incubation for 24h with TPA-Mit (0 μM, 0.2 μM, 0.5 μM, 1 μM, 1.5 μM, 2 μM).

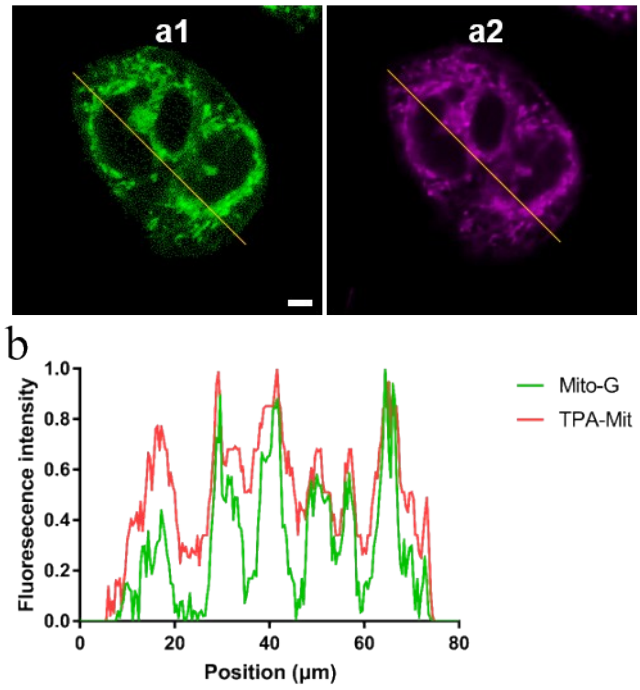

Fig. S6. The cross section through the image

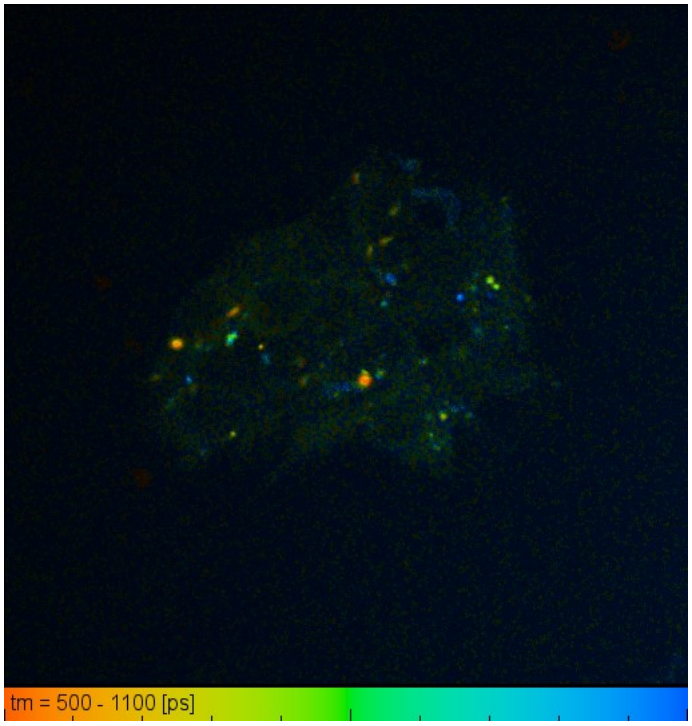

Fig. S7. Fluorescence lifetime image of cellular autofluorescence. excitation power to about 10 mW after imaging objective.

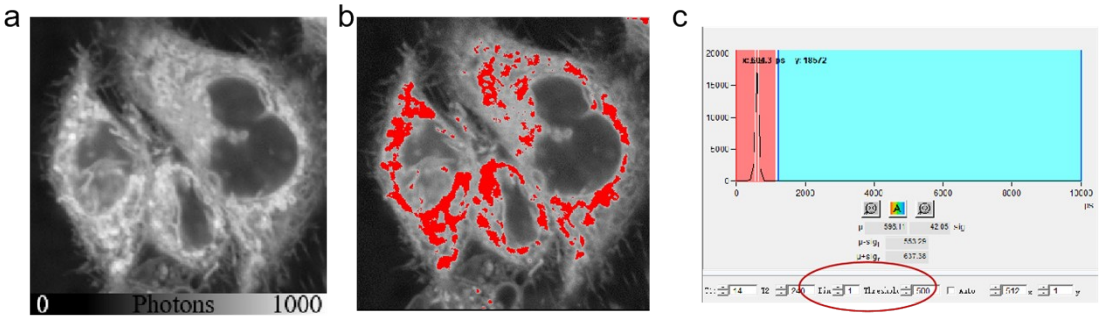

Fig. S8. Section selection based on the threshold of photon count. a. Photon count of SKOV-3 stained by TPA-Mit (0.2 μM). b. Section separation result when the threshold was set 500 photons (>500 photons for the red region). c. User interface of the *SPC image* (Becker & Hickl GmbH, Germany).

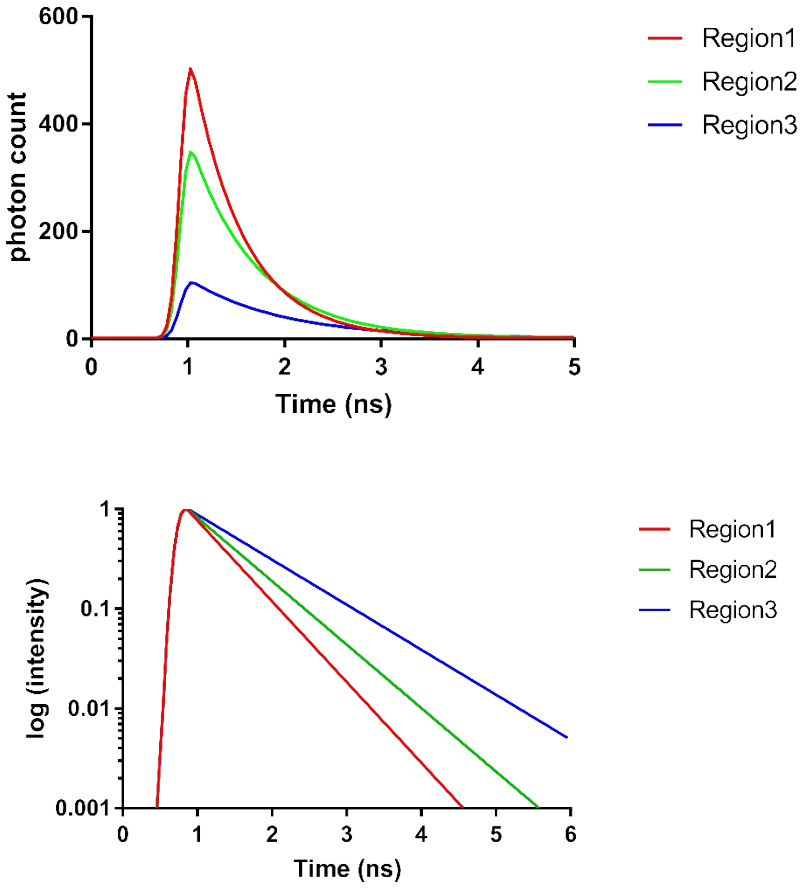

Fig. S9. The representative decays in three regions

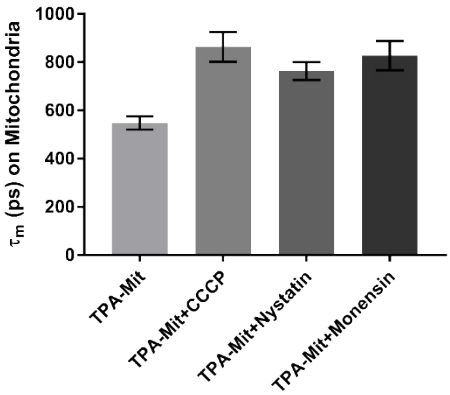

Fig. S10. Fluorescence lifetime change of TPA-Mit after the stained SKOV-3 cells were treated with Carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 2 μM), Monensin (10 μM) and Nystatin (10 μM) for 24h.