Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

Short-wave infrared emitted/excited fluorescence from carbon dots and preliminary applications in bioimaging

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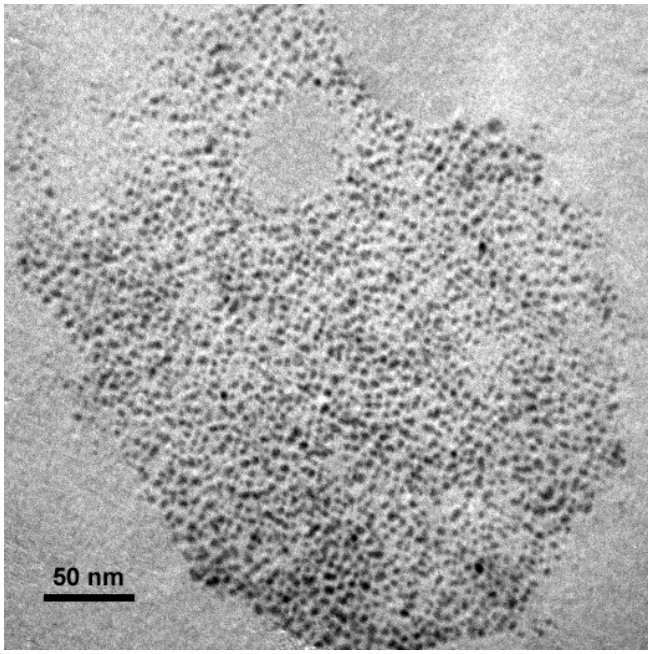

Figure S1. A typical TEM image of FCDs.

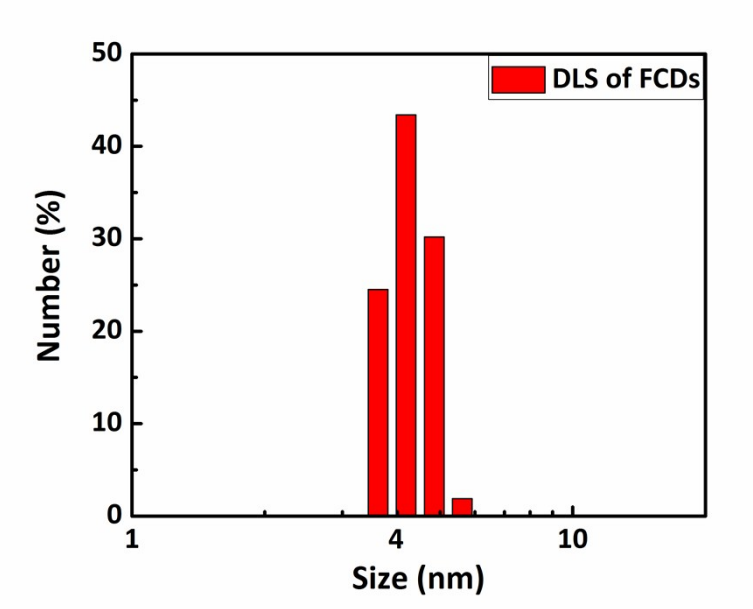

Figure S2. Hydrodynamic size of FCDs measured by dynamic light scattering.

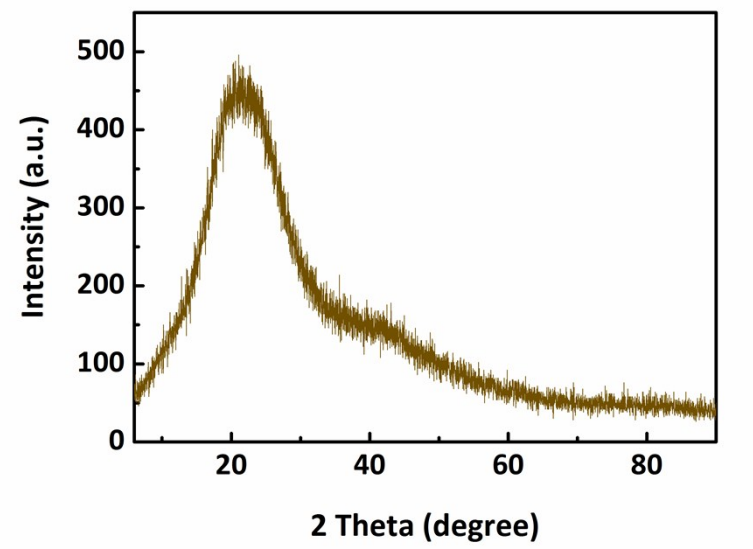

Figure S3. XRD patterns of the FCDs.

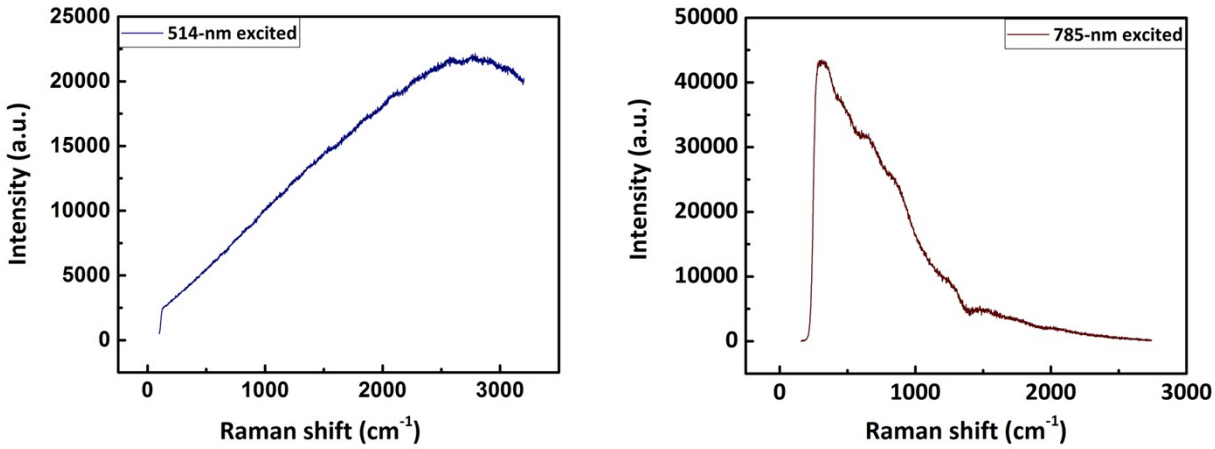

Figure S4. Raman spectra of FCDs under the 514-nm and 785-nm excitation.

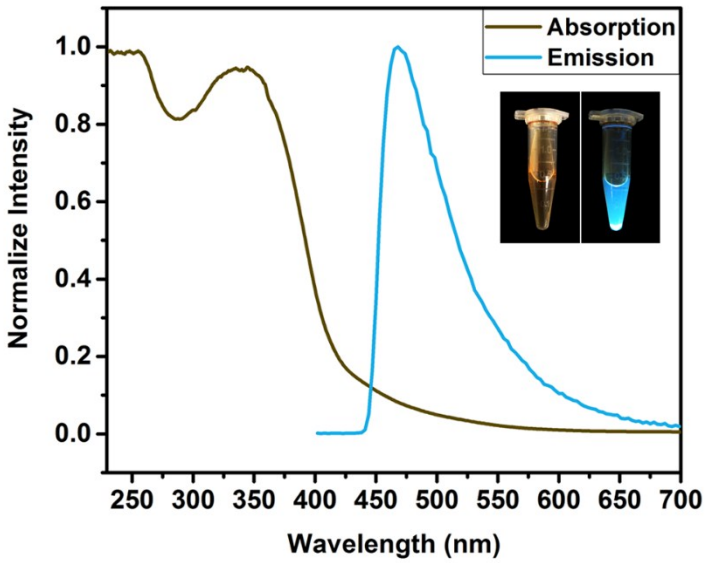

Figure S5 Absorption and emission spectra of aqueous dispersion of FCDs. Excitation wavelength: 405 nm. Inset: Bright field and fluorescence photographs of FCDs in DI water.

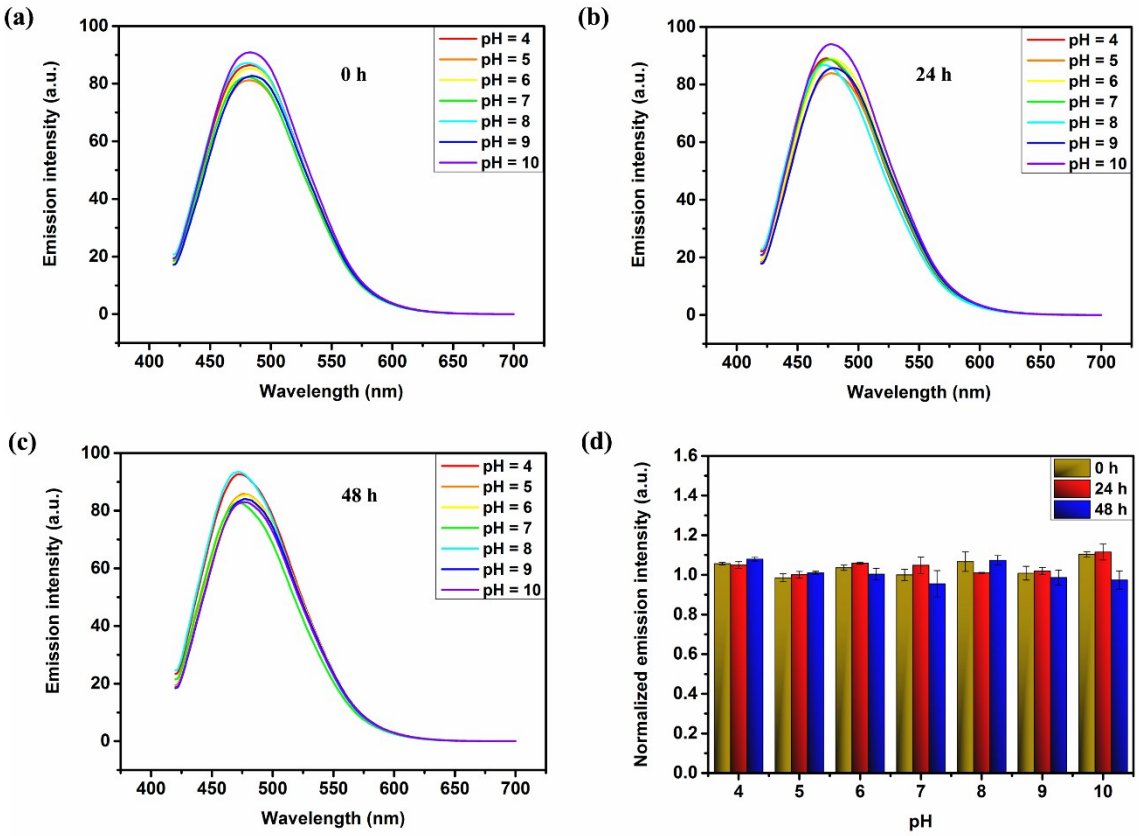

Figure S6 (a) Emission spectra of freshly synthesized FCDs in aqueous dispersions with different pH values. (b) Emission spectra of FCDs in aqueous dispersions with different pH values, 24 hours post treatment. (c) Emission spectra of FCDs in aqueous dispersions with different pH values, 48 hours post treatment. (d) Emission intensity comparison between freshly synthesized FCDs, FCDs after 24-hour treatment and 48-hour treatment in different pH values. Excitation wavelength: 405 nm.

Cell culture and treatment:

HeLa cells and NCl-H1688 cells (human cancer cell lines) were cultivated in Dulbecco minimum essential media (DMEM) with 10 % fetal bovine serum (FBS), 1 % penicillin and 1

% amphotericin B. One day before the treatment, cells were seeded in 35 mm cultivation dishes at a confluence of 70-80 %. The cell incubation process with FCDs lasted for 2 h at 37 ºC with 5 % CO_2. The cells were then washed thrice with phosphate buffered saline (PBS) and directly imaged using a commercially available upright two-photon fluorescence scanning microscope (BX61+FV1000, Olympus).

In vitro cytotoxicity was measured by performing MTT assays on NCl-H1688 cells. Cells were seeded into a 96-well cell culture plate at 5×10^3/well and cultured at 37 ºC with 5% CO_2 for 24 h. 200 μL fresh MEM with different concentrations of FCDs (50, 100, 150, 200, 250, 300 μg/mL) were then added into the wells. The cells were subsequently incubated for 48 hours at 37 ºC with 5% CO_2. Then, MTT (20 μL/well, 5 mg/mL) was added to each well and the plate was incubated for additional 4 hours at 37 ºC with 5% CO_2. MTT was reacted with succinatede hydrogenase in the mitochondria of living cells to form formazan (with characteristic absorption at 450 nm). The dead cells were then removed with the medium, and 200 μL dimethyl sulfoxide (DMSO) was added to per well to resolve the formazan, and optical density (OD) at 570 nm of each well was monitored by an enzyme-linked immune sorbent assay (ELISA) reader. The following formula was used to calculate the cell viability:

Cell viability (%) = (mean $OD_{570\ nm}$ of treatment group - mean $OD_{570\ nm}$ of blank group)/ (mean $OD_{570\ nm}$ of control group - mean $OD_{570\ nm}$ of blank group) × 100%

where blank group means group without cells or FCDs; control group means group with cells but without FCDs, while other procedures are the same as treatment group described above.

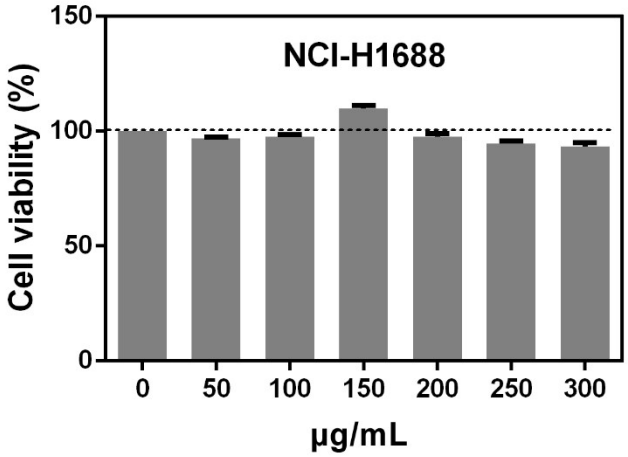

Figure S7. Viability of NCl-H1688 cells after incubated with various concentrations of FCDs.

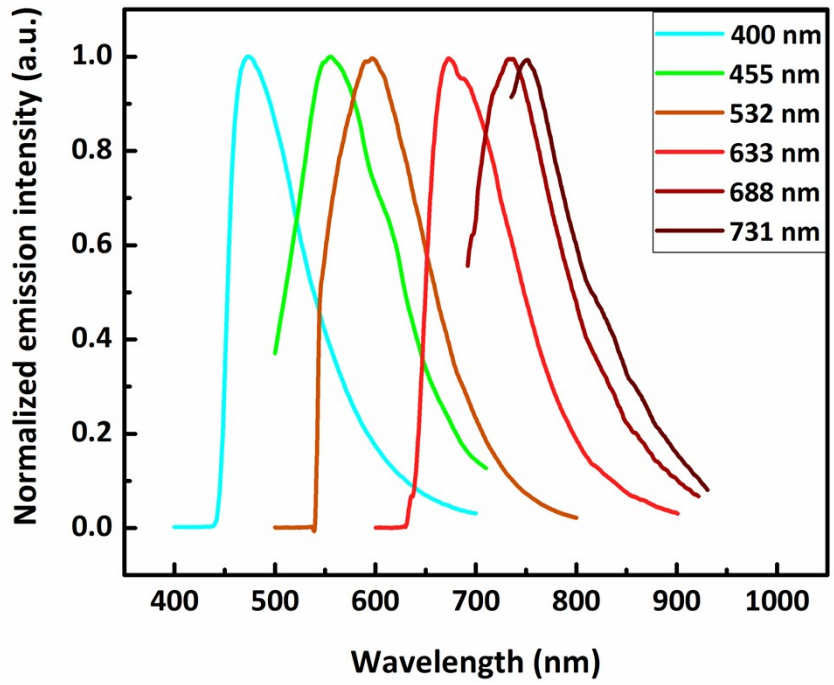

Figure S8. Fluorescence spectra of FCDs in aqueous dispersion, excited by the 400-nm, 455-nm, 532-nm, 633-nm, 688-nm and 731-nm CW light.

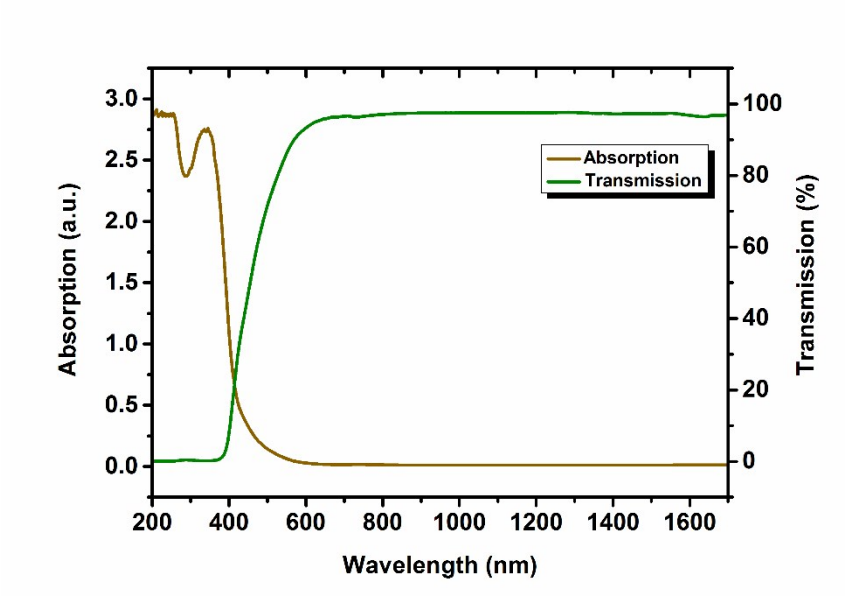

Figure S9. Absorption and transmission spectra of FCDs.

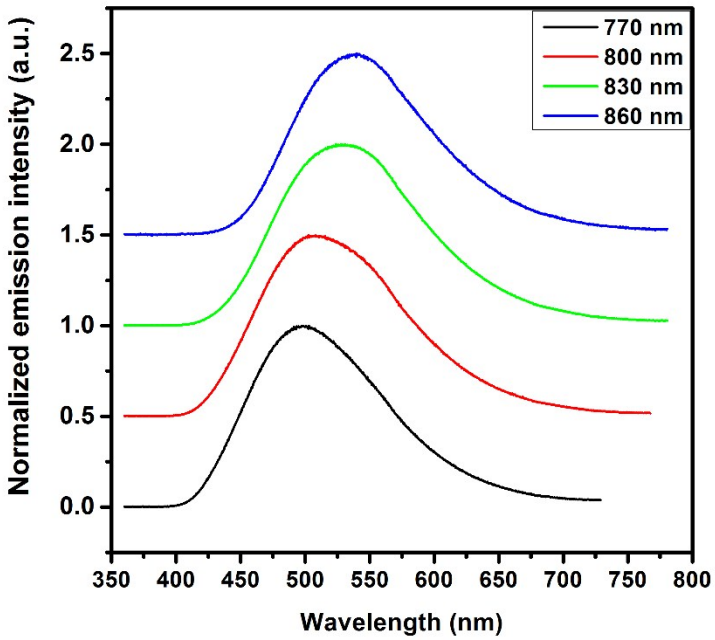

Figure S10. Two-photon fluorescence spectra of FCDs in aqueous dispersion, excited by the 770-nm, 800-nm, 830-nm and 860-nm fs laser.

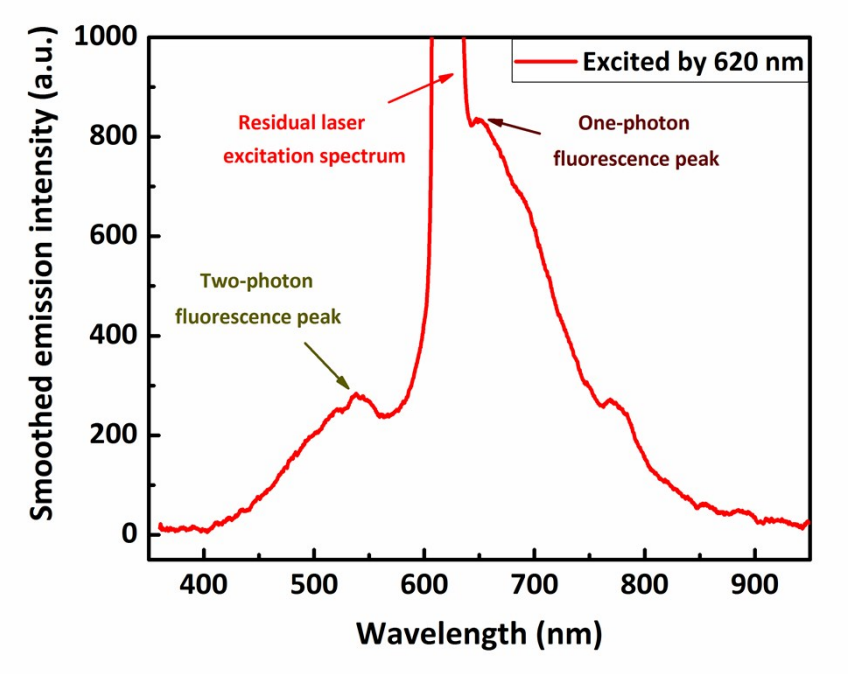

Figure S11. Fluorescence spectra of FCDs excited by a 620-nm fs laser.

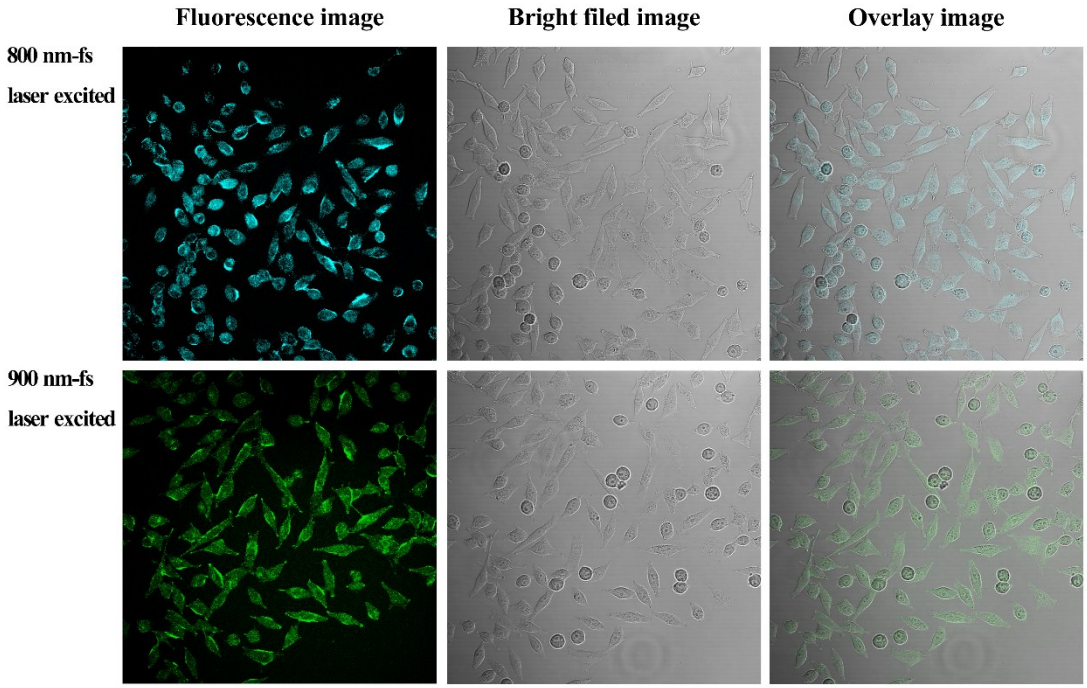

Figure S12. Two-photon fluorescence images of HeLa cells stained with FCDs, under the fs excitation of 800 nm and 900 nm.

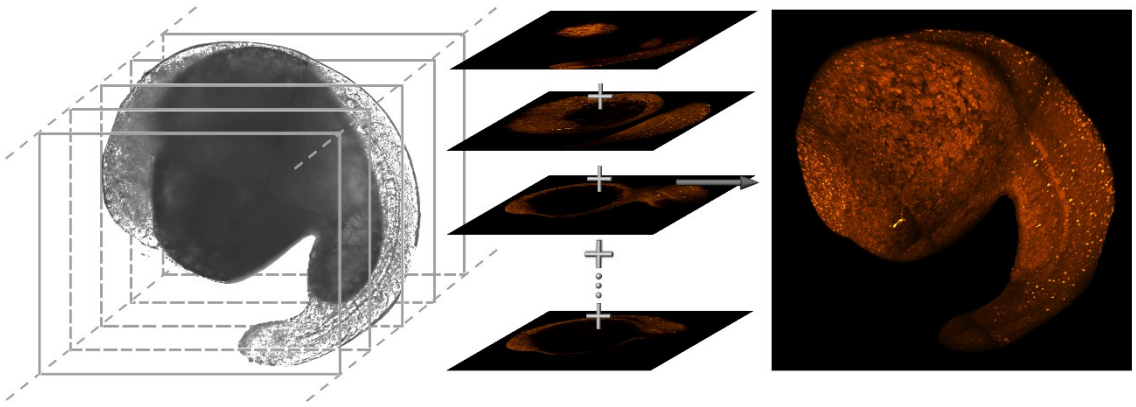

Figure S13. Schematic illustration of two-photon fluorescence signal-stacked imaging of zebrafish embryos, under the fs excitation of 1040 nm.

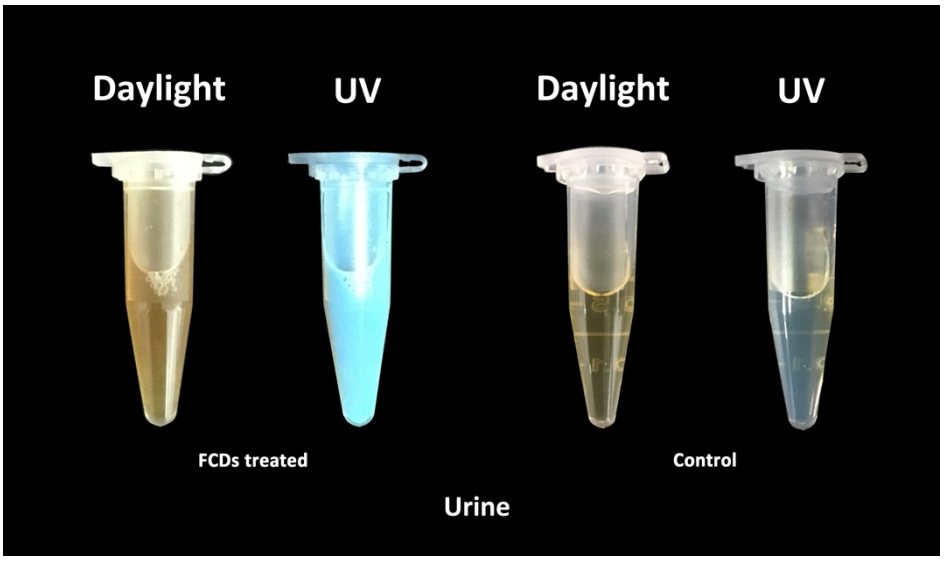

Figure S14. Images of the urine samples collected from the FCDs injected mouse, under daylight and UV light.

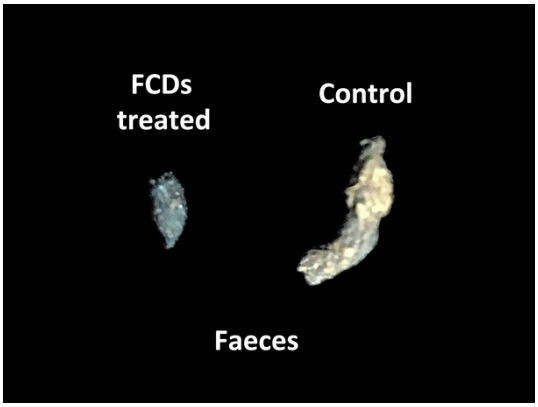

Figure S15. Images of the faeces sample collected from the mice with and without FCDs treatment, under UV light irradiation.

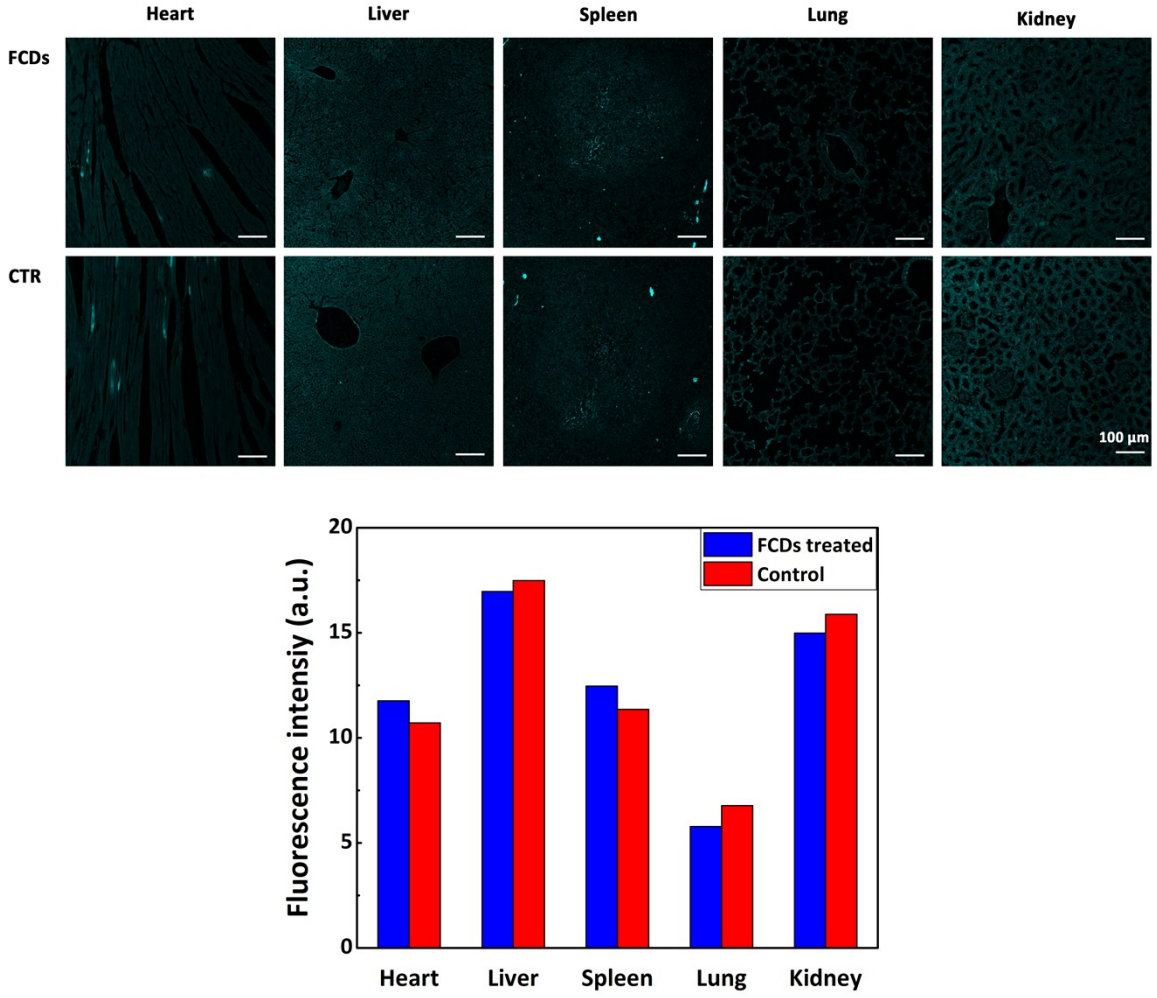

Figure S16. Fluorescence images and intensities of the sliced main organs of FCDs treated mice 30 days post injection.

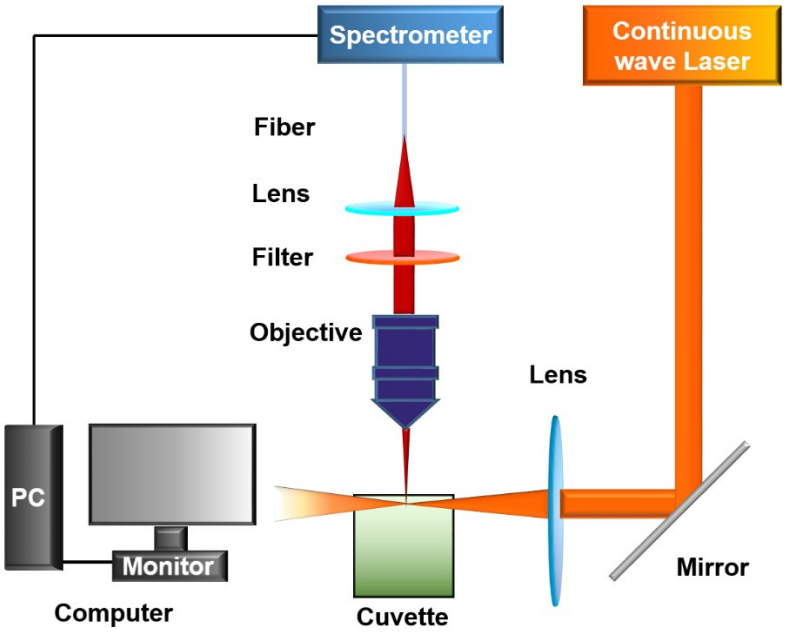

Figure S17. Schematic illustration of the setup for measuring one-photon fluorescence spectra of FCDs.

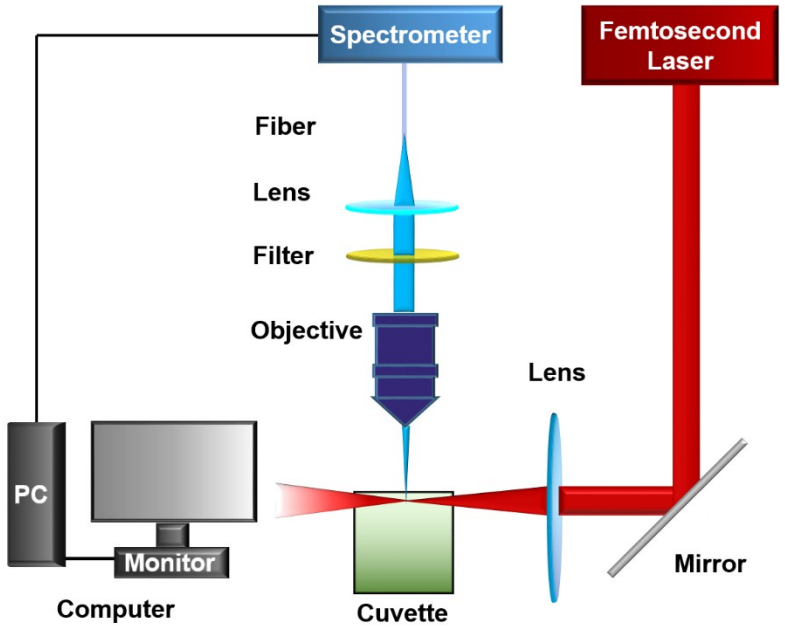

Figure S18. Schematic illustration of the setup for measuring two-photon fluorescence

spectra of FCDs.

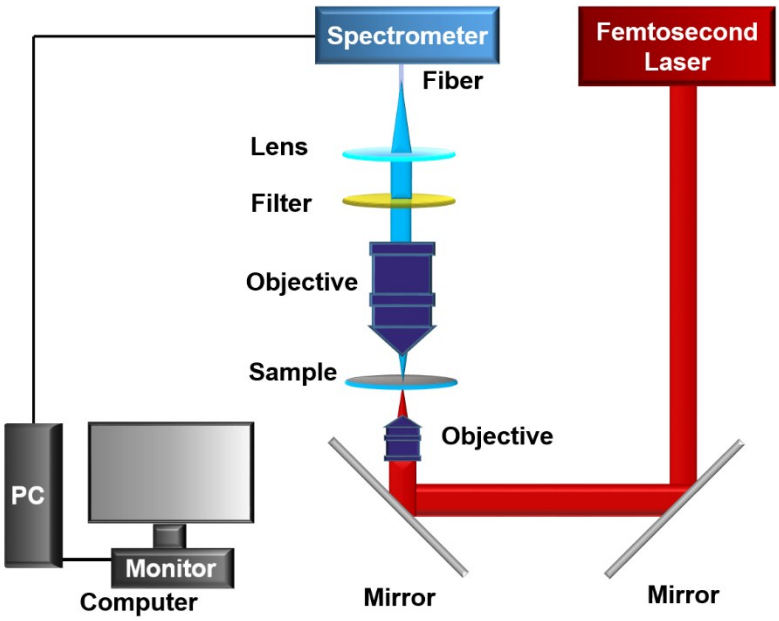

Figure S19. Schematic illustration of the setup for measuring THG spectrum of FCDs.

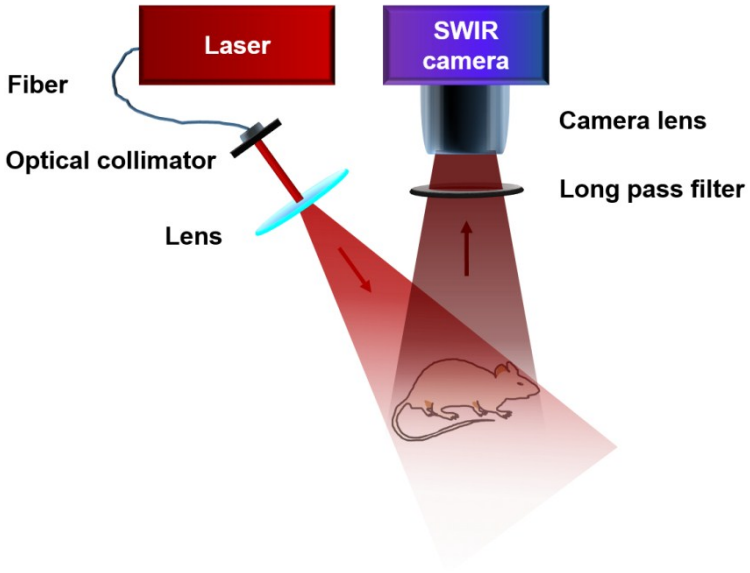

Figure S20. Schematic illustration of the setup for SWIR fluorescence bioimaging.

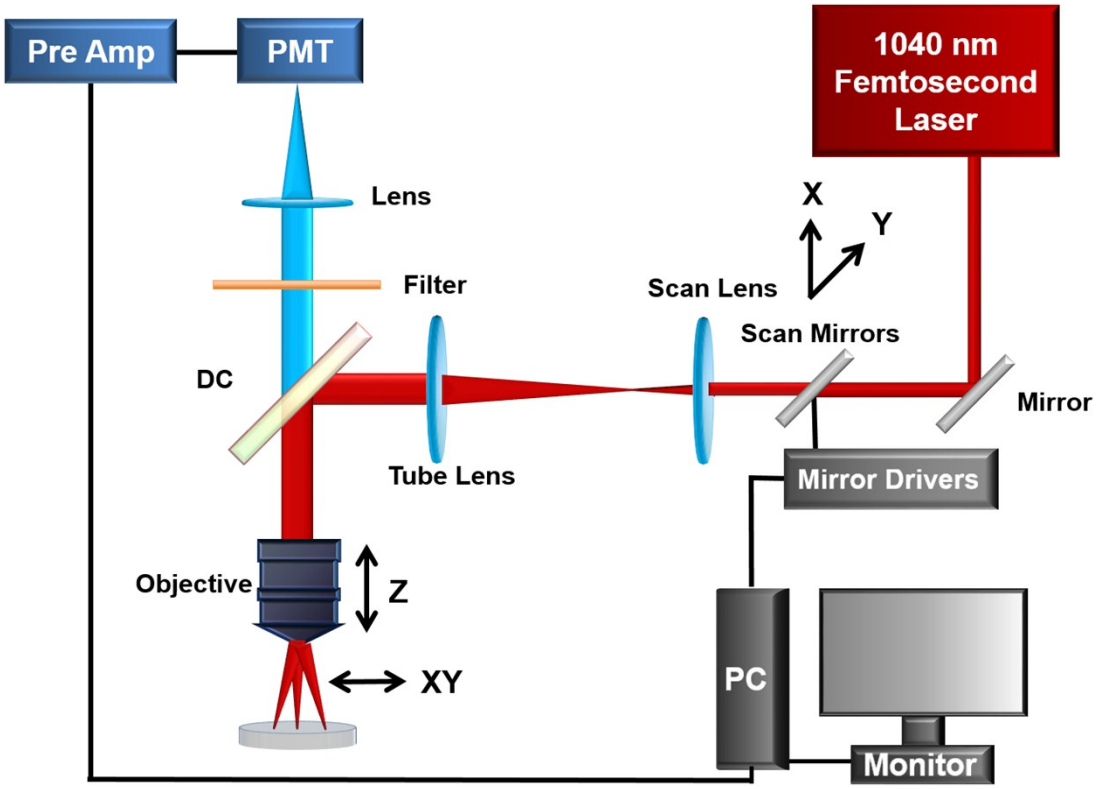

Figure S21. Schematic illustration of the two-photon fluorescence imaging system equipped with a 1040-nm fs laser.