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Protocol

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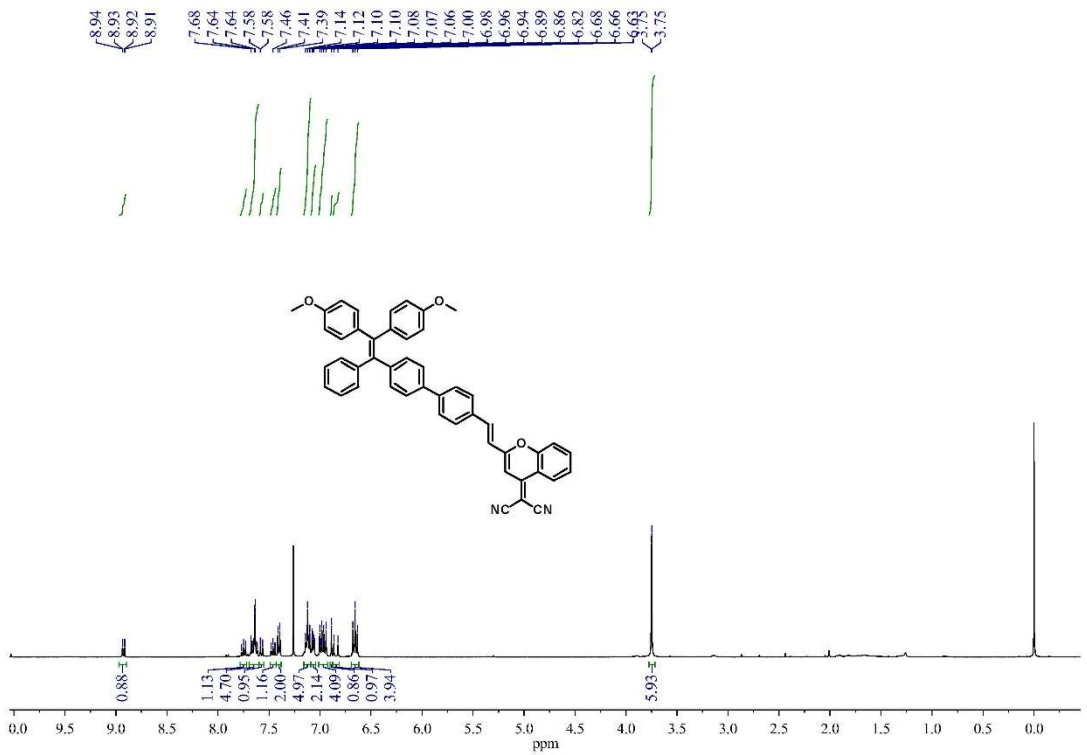
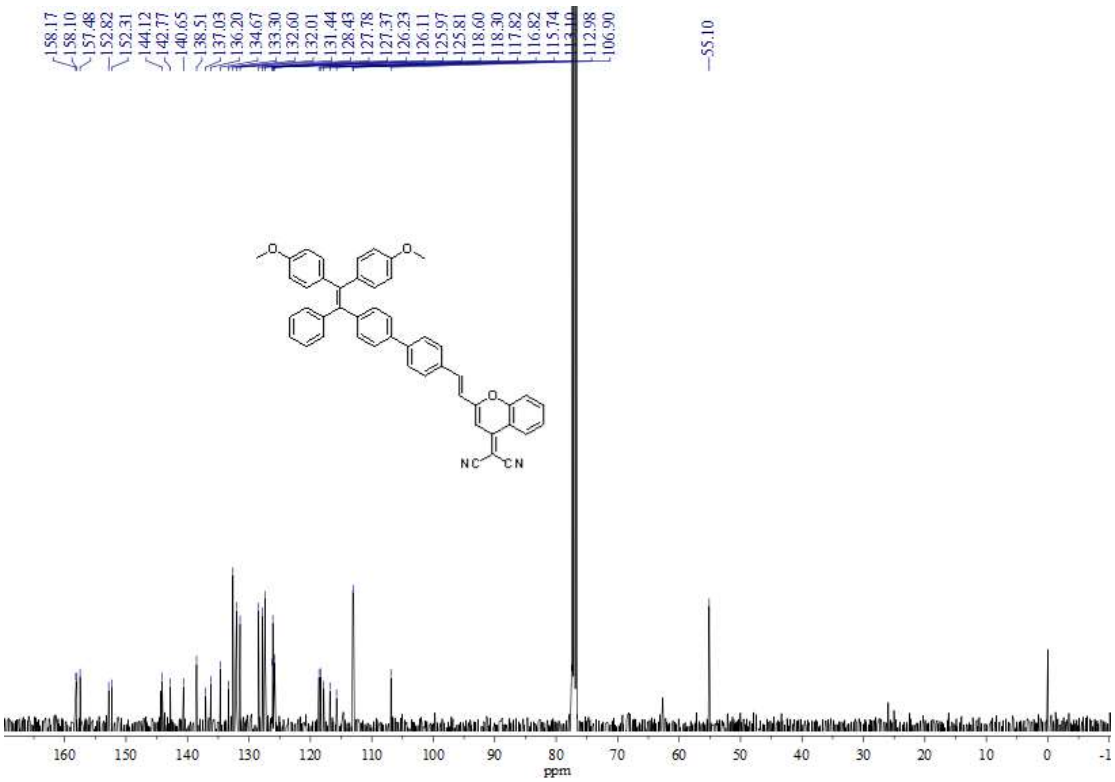
Preparation of AIEgen-based near-infrared afterglow luminescence nanoprobes for tumor imaging and image-guided tumor resection

In the format provided by the authors and unedited

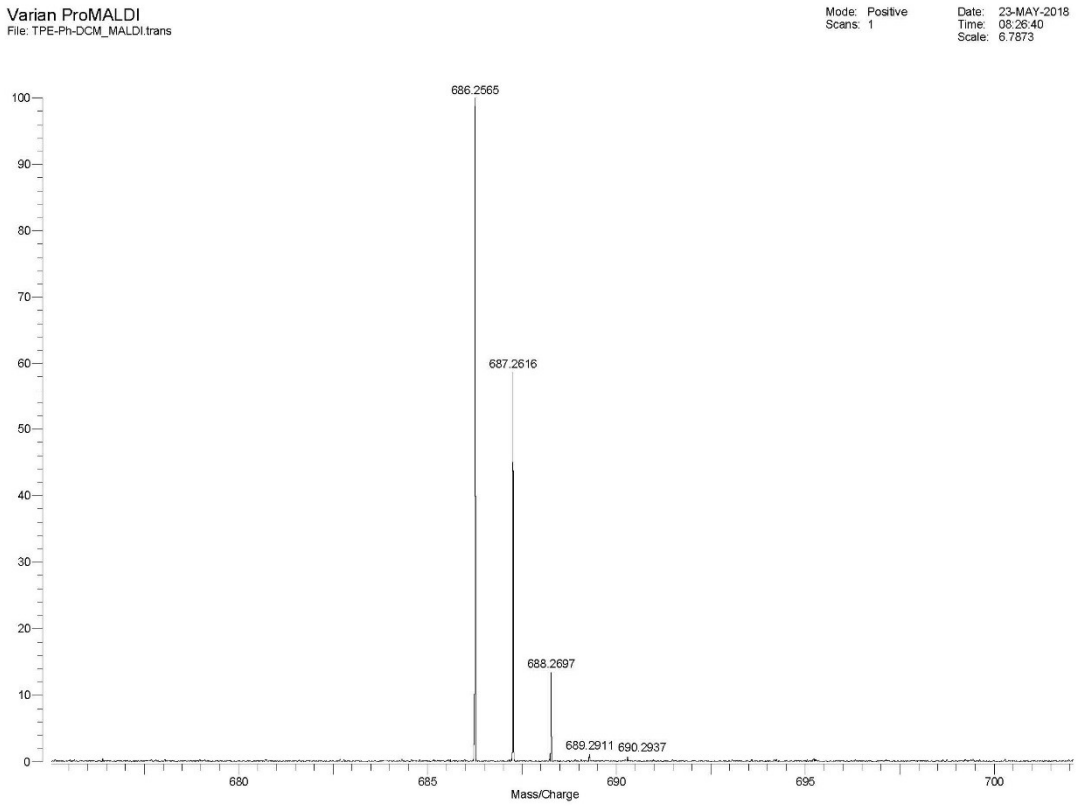
Supplementary Figures



Supplementary Fig. 1. ^{1}H NMR spectrum of TPE-Ph-DCM. Adopted from ref[1].



Supplementary Fig. 2. ^{13}C NMR spectrum of TPE-Ph-DCM. Adopted from ref[1].



Supplementary Fig. 3. HRMS spectrum of TPE-Ph-DCM. Adopted from ref[1]. Copyright 2019, American Chemical Society.

Supplementary Methods

Procedure for building the peritoneal carcinomatosis-bearing mouse model.

Materials:

Biological Materials

- Murine breast cancer 4T1 cell (4T1 cell, ATCC, cat. no. CRL-2539, RRID: CVCL_0125)

  ! CAUTION Cell culture needs to be performed aseptically and regularly checked for mycoplasma contamination.

- Mice (BALB/c, female, 6-weeks-old) purchased from Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China)

  ! CAUTION: All animal studies in this protocol were performed in compliance with the guidelines set by the Tianjin Committee of Use and Care of Laboratory Animals, and the overall project protocols were approved by the Animal Ethics Committee of Nankai University. When using this protocol, the handling of animals must also conform to relevant Institutional and National regulations.

Reagents

Fetal bovine serum (BioInd, cat. no. 04-001-1ACS)

Penicillin-Streptomycin (Gibco, cat. no. 15140-122)

RPMI-1640 medium (Gibco, cat. no. 11875093)

Trypsin (Gibco, cat. no. 15090046)

PBS buffer (Solarbio, cat. no. P1020)

Equipment

Cell culture dish (NEST, cat. no. 706001)

15-mLsterile centrifuge tube (Corning, cat. no. 430791)

Cell counting chamber (Hausser scientific, cat. no. 3100)

Cell counting chamber slides (Brand, cat. no. BR723015)

Culture microscope (OLYMPUS, CKX53)

Cell culture incubator (Thermo Fisher Scientific, HERACELL 240 i)

Clean bench (SHINVA, CJV1000-Y)

Procedure: Building the peritoneal carcinomatosis-bearing mouse model Timing 7 days.

1. Culture 4T1 murine breast cancer cells in a cell culture dish using RPMI-1640 medium supplemented with 10% fetal bovine serum, 10 U/mL penicillin, and 10 mg/mL streptomycin. Place the dish in a cell incubator (37 °C, 95% humidity, 5% CO_2) until the cells grow to 70-80% confluency.
2. Dissociate the adherent 4T1 cells by adding 1 mL of trypsin and incubate for 5 min at 37°C.
3. Add 2 mL of RPMI-1640 medium supplemented with 10% fetal bovine serum to neutralize trypsin activity.
4. Aspirate the liquid in the dish with a pipette to carefully resuspend the cells and detach them.
5. Transfer the cell suspensions into a 15-mL sterile centrifuge tube and centrifuge the cells at ~266 g for 5 min to obtain a cell pellet.
6. Carefully discard the supernatant. Add 1 mL of sterile 1 × PBS and resuspend the cells evenly by pipetting up and down.
7. Estimate the cell density using a cell counting chamber under a microscope. Adjust the density of cell suspension to 3×10^6/mL, adding the required volume of complete RPMI-1640 medium.
8. Aspirate 100 μL of the cell suspension with a disposable insulin syringe, point the syringe needle up, flick the syringe several times, and slowly press the syringe to squeeze out the bubbles.
9. Hold the mouse with the left hand, position the mouse head down 45 degrees, hold the syringe with the right hand, quickly insert the needle into the mouse abdominal cavity, and slowly inject the cell suspension.

   CRITICAL STEP The mouse can be grasped with either the left hand or the right hand, and the mouse should be grasped tightly. Just insert the needle into the

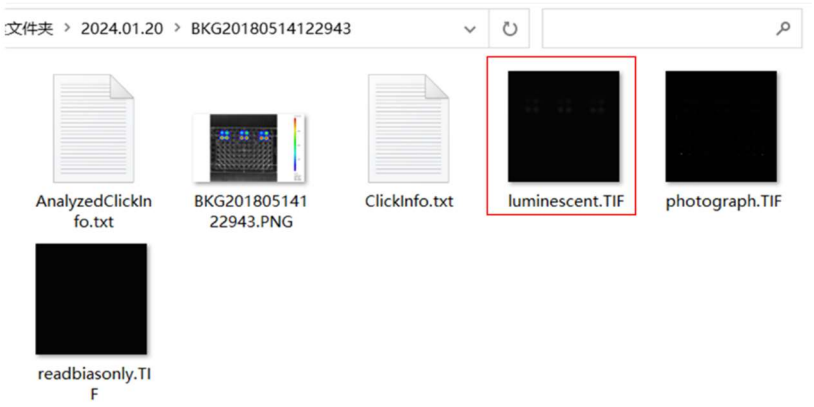
abdominal cavity; avoid inserting it all the way into the abdominal cavity to prevent the viscera or intestines from being stabbed.

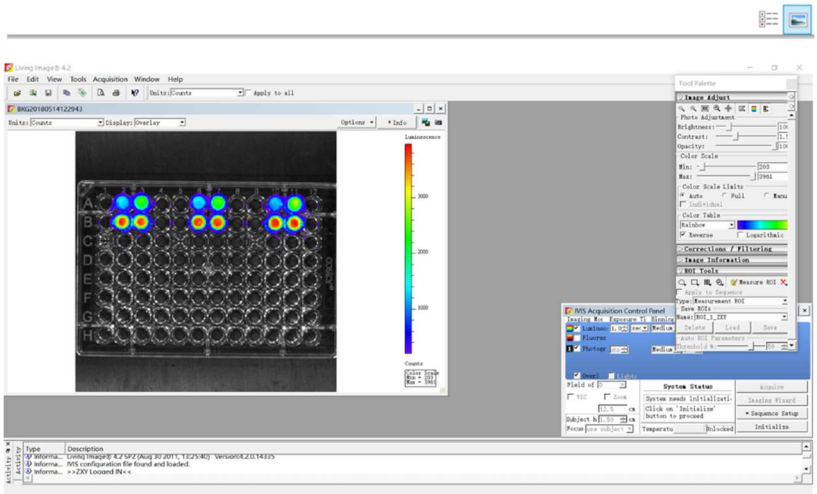
10. Withdraw the needle and clean the abdomen with sterile alcohol cotton. Place the mouse in a recovery cage.
11. Repeat Steps 8 and 9 to build the required number of tumor-bearing mice. After 5 days, the peritoneal carcinomatosis-bearing mouse model is built and can be used for imaging experiments.

Procedure for Imaging Data Analysis

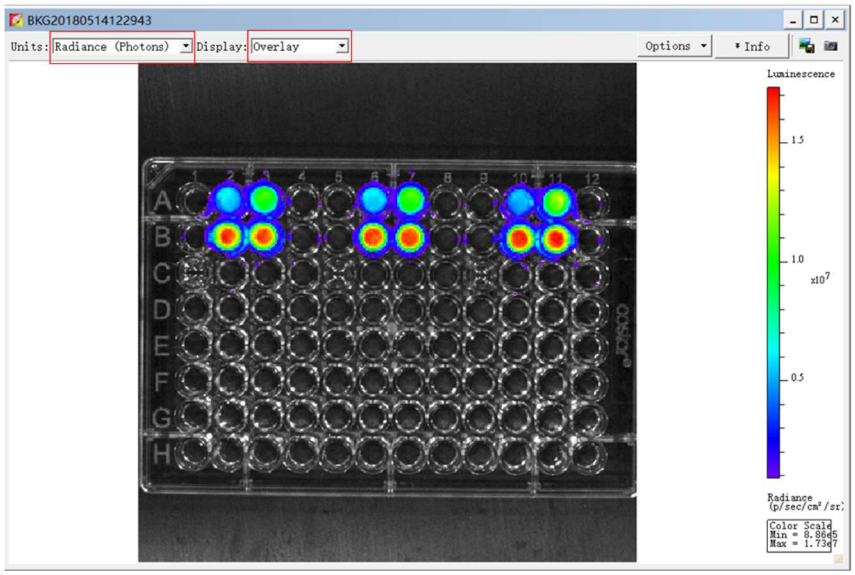
CRITICAL: Here, we provide step-by-step guidelines for imaging data analysis, using the data from Fig. 6a as an example.

1. Open Living imaging software (PerkinElmer, version: Living Image 4.2), and import the data file (luminescent.TIF). Take the afterglow image processing process at the time point of 2 h in Fig. 6a as an example.

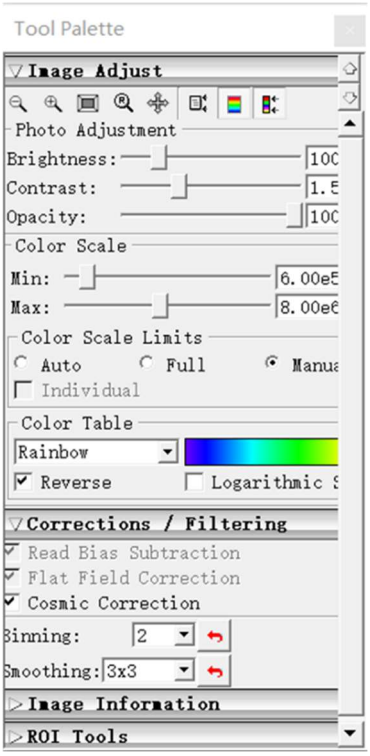




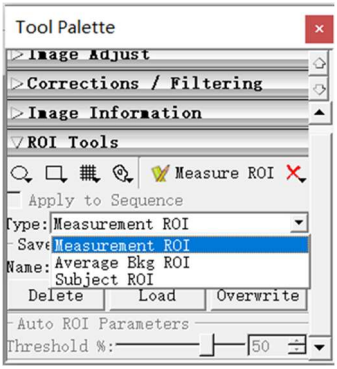
2. Set the “Units” to “Radiance (Photons)” and the “Display” to “Overlay”.



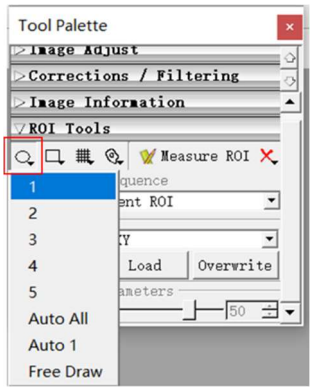
3. Open the “Tool Palette” window, and set parameters in the “Image Adjust ” and “Corrections/Filtering” sections to be brightness: 100, contrast: 1.5, opacity: 100, Min: 6×10^5, Max: 8×10^6, Binning=2, Smoothing: 3×3.



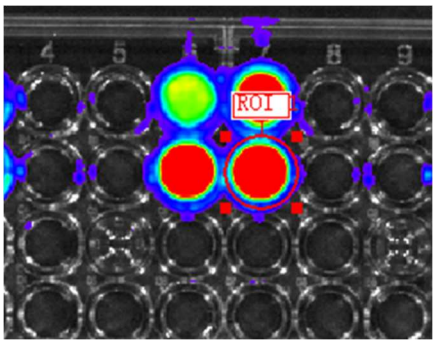
4. Click “ROI Tools”, select Measurement ROI from the “Type” drop-down list.



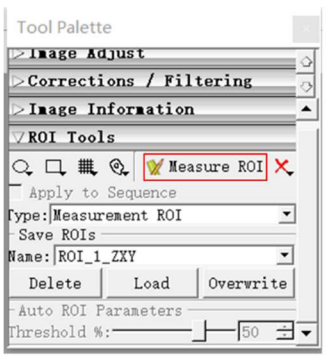
5. Click the circle button in “ROI Tools” and select “1” from the drop-down.

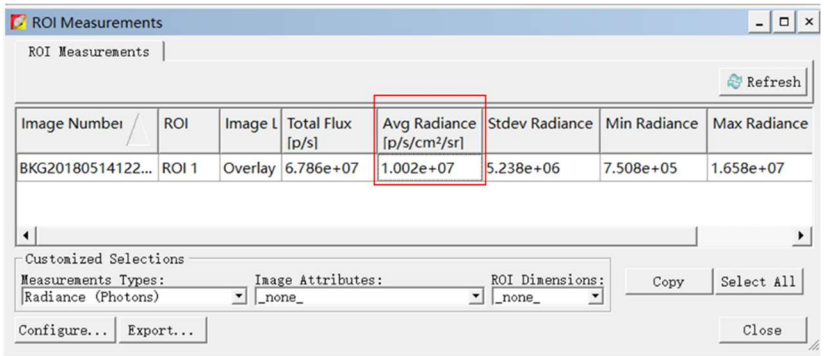


6. Adjust the position of the ROI circle size and select the correct area of the 96-well plate.

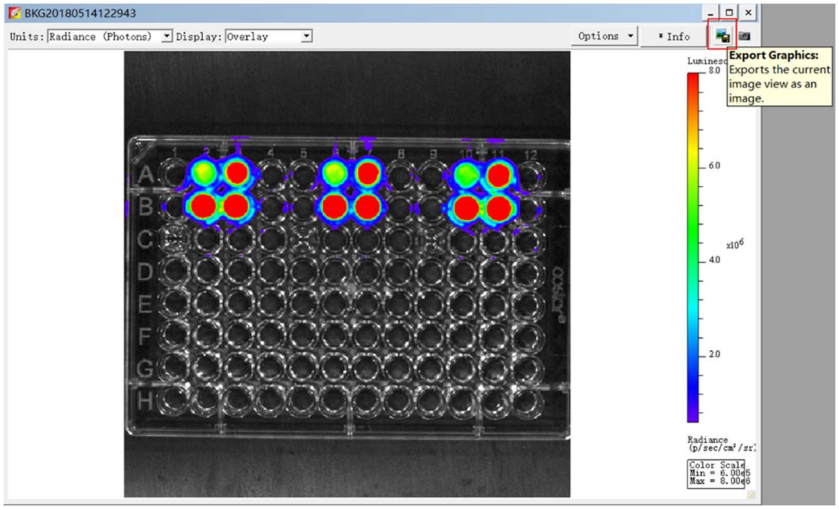


7. Click the “Measure ROI” button on “Tool Palette” to open the “ROI measurements” window.





8. In the “ROI Measurements” window, record quantitative value. The value in Avg Radiance [p/s/cm^2/sr] is the average afterglow intensity of interest.
9. Click the “Export Graphics” button to save the processed afterglow image.



References

1 Ni, X. et al. Near-infrared afterglow luminescent aggregation-induced emission dots with ultrahigh tumor-to-liver signal ratio for promoted image-guided cancer surgery. *Nano Lett.* **19**, 318-330 (2019).