Acknowledgment examples

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Materials and Methods Example (optical imaging)

Example #1: Confocal Z-stack imaging

Fluorescent images were obtained using a Carl Zeiss LSM510 META (Germany) laser scanning confocal microscope with a Plan Apochromat 63x 1.4NA oil immersion objective. Images were collected as Z stacks with a Z step size at 0.43µm and z-stacks were rendered for 3D reconstruction with ZEN software (Carl Zeiss, Germany).

Example #2: Live cell imaging (widefield)

For live cell observation, we used a ZEISS Axio Observer microscope (Carl Zeiss, Germany) equipped with an environmental control chamber. GFP and mCherry were detected with a BP 515-565nm band-pass filter and a LP 590nm long-pass filter, respectively. Phase contrast and fluorescent images were acquired with a Plan Neofluar 10x 0.3NA objective in 10 different microscopic fields at 5 minute interval for 24hrs. Quantitative measurements of GFP and mCherry fluorescence intensity at each time points were carried out with MetaMorph software (Version 7.7.11, Molecular Device, US).

Example #3: Live cell imaging (spinning disc confocal)

Live imaging of cultures was performed using a Perkin-Elmer UltraView ERS spinning disk system (PerkinElmer Inc.) attached to a ZEISS Axio Observer microscope with an EM CCD camera (Evolve512, Photometrics, US). Images were acquired in stacks of 10 optical sections along z-axis (z-stacks) at interval of 1um with a Plan Apochromat 20x 0.8NA objective. Images were taken at 1 minute interval for 2hrs and processed with MetaMorph software (Version 7.7.11, Molecular Device, US).

Materials and Methods Example (Flow Cytometry)

Example #1: Cell analysis

Single cell suspensions were analyzed using FACS CantoII flow cytometer (BD Biosciences, San Jose, CA, USA) equipped with a 488nm solid state laser. The fluorescence of GFP was detected with

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an emission filter of 530/30nm. A gate was drawn in the Side Scatter (SSC) versus Forward Scatter (FSC) plot to exclude debris and aggregated cells. Twenty thousand events of gated population were collected in each run. Data analysis was performed using FlowJo 7.6.1 software (Tree Star, Inc., Ashland, OR, USA).

Example #2: Cell sorting

The sorting of the labeled cell suspension was performed using FACSAria SORP cell sorter (BD Biosciences, San Jose, CA, USA). The GFP and PE positive cells were gated and sorted using the four-way purity mode and the purity of sorted cell populations was >96%.

<u>Materials and Methods Example (Animal Imaging)</u>

Example #1: Whole animal fluorescence imaging

Mice were imaged at different time points using the Maestro In-Vivo Imaging System (CRI, Inc.). Images were acquired with a band-pass excitation filter from 616 to 661 nm and a long-pass emission filter over 675 nm, with exposure times between 300 ms and 1 s. A liquid-crystal tunable wavelength filter was set for collection of emission images from 680 to 720 nm in 10 nm increments. Spectral fluorescence images based on autofluorescence and Cy5.5 spectra were obtained and spectra unmixed using Maestro software (CRi, Inc.). Mice were sacrificed immediately after completion of imaging. Surgery was then done to dissect the tumors and enable ex vivo optical imaging, using the same Maestro settings.

Example #1: Whole animal bioluminescence imaging

Mice were anesthetized by intraperitoneal (IP) injection of 25 mg sodium pentothal (Nembutal)/kg BW. Ten minutes before imaging, an intraperitoneal injection of 15mg/kg body weight D-luciferin firefly potassium salt (Xenogen) was administered to the anesthetized mice. Whole body images of bioluminescent signals were acquired for 1–5 min in vivo imaging system Xenogen IVIS 100 (Xenogen, Alameda, CA). Regions of interest (ROI) were manually selected, and the results were quantified as average radiance of photons emitted per second and area (p/s/cm2) by using the Living Image 2.50.2 software (Xenogen, Alameda, CA).

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