Experimental

Sample Preparation: We employed an implantable drug-delivery microdevice, adapted from reference 18, to deliver micro doses of the anti-cancer drug doxorubicin into melanoma or breast tumors in nude mice (Fig 1). This allowed us to avoid the inconsistency and heterogeneity of drug distribution which is associated with systemic dosing of anti-cancer drugs. It distributes over a region of approximately 400 μm in diameter within 24 hours. The drug was allowed to react with the native tumor tissue within this region of exposure while leaving the remainder of the tumor unexposed. The intratumor concentration of doxorubicin in this release system has been shown to closely match those intratumor drug levels that are achieved clinically during traditional systemic drug administration [18]. Tumor sections containing both exposed and non-exposed regions were freshly excised from animals, sectioned and immediately imaged using Raman spectroscopy, Fig 2.

Raman spectroscopy: A previously reported custom-built NIR confocal Raman microscopy system was used for the experiments [19,20]. 785nm wavelength from Ti: Sapphire laser (3900S, Spectra-Physics) was used as an excitation source. The laser beam is focused onto the sample, and backscattered light is collected by the same objective lens. Raman signal is collected after series of Rayleigh rejection filters by a collection fiber which is connected to a high-throughput imaging spectrograph (Holospec f/1.8i, Kaiser Optical Systems) with a thermoelectric-cooled, back-illuminated, and deep depleted CCD (PIXIS: 100BR_eXcelon, Princeton Instruments). The laser beam was scanned by the galvanometer mirrors (CT-6210, Cambridge Technology). Freshly excised tumor sections were placed on top of quartz coverslip (043210, Alfa Aesar) for Raman imaging. We used 60 mW incident laser power for Raman mapping on the sample and 10s integration time per pixel for 10 × 10 pixels, leading to a total imaging time of 17 min.

Statistical Analysis: Quantitative analysis was performed by decomposing the acquired Raman spectra from the tissue specimens as linear super positions of basis components, DNA, albumin, actin, collagen, triolein, and phosphatidylcholine. The normalized spectra from these agents were acquired from pure chemicals. They were used as the basis spectra for analysis (Fig 3) and represented as a vector. Each acquired spectrum was represented by six fitting coefficients, representing the relative amounts of basis components. The ordinary least squares fitting method was used for biochemical decomposition of the tissue spectra (Fig 3) over the fingerprint region (600-1800 cm^{-1}). Acquired Raman spectra from tissues were normalized and decomposed as a summation of basis components as previously described [20,21]. To avoid data overfitting, the fitting process was carefully monitored by restricting the fitting coefficient to non-negative values.