

Supporting Information

Label-free Raman spectroscopic imaging monitors the integral physiologically relevant drug responses in cancer cells

Samir F. El-Mashtoly,^{§,‡} Hesham K. Yosef,^{§,‡} Dennis Petersen,[§] Laven Mavarani,[§]
Abdelouahid Maghnouj,[†] Stephan Hahn,[†] Carsten Kötting,[§] and Klaus Gerwert^{*,§}

[§]Department of Biophysics and [†]Department of Molecular GI-Oncology, Clinical Research Center, Ruhr-University Bochum, 44780 Bochum, Germany

*To whom correspondence should be addressed. Department of Biophysics, Ruhr-University Bochum, 44780 Bochum, Germany. Tel: +49-234-32-24461. E-mail: gerwert@bph.rub.de.

[‡]Both authors contributed equally to this work.

Cytotoxicity Assay

Working solutions of panitumumab were prepared in cell culture media. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Sigma-Aldrich, (München, Germany). MTT (yellow) is reduced to purple formazan by mitochondrial reductases in living cells; therefore, it is a useful indicator of cell viability and proliferation. The MTT assay was performed as described previously with minor modification (66). Cells were grown in 24-well plates at a density of 2×10^3 cells per well in DMEM medium containing all the supplements mentioned above. After 24 h of initial cell attachment, the plates were washed with 100 μ l PBS per well, and incubated with various panitumumab concentrations.

After 12 h of treatment, cells were rinsed with PBS and 100 μ L of fresh medium (without supplements) was added to each well. Then, MTT (5 mg/ml) was added to each well, and the plates were incubated at 37°C in a 10% CO₂ humidified incubator for 4 h. The medium was discarded, and cells were washed with 100 μ L of PBS. Then, 100 μ L of DMSO was added to each well to extract the dye. The plates were then shaken, and absorbance was detected at 570 nm using a microplate reader (TECAN Sunrise, Austria). Four replicate wells were used for each exposure. The viability in each sample was normalized to that of the control sample.

Western Blot Analysis

Cells were harvested in a buffer containing phosphatase inhibitor mixture II (Sigma-Aldrich, München, Germany) and protease inhibitor cocktail (Roche, Mannheim, Germany), were resolved by SDS-PAGE using 10% polyacrylamide gels, and transferred to Immobilon-P (Millipore, Hessen, Germany) membranes. The membranes were then incubated with antibodies to phosphorylated ERK1 and ERK2 (p-ERK, Cell Signaling,

Danvers, USA) and total ERK1 and ERK2 (total ERK, Cell Signaling, Danvers, USA). Antibodies were detected with the appropriate anti-mouse horseradish peroxidase-conjugated secondary antibody enhanced by chemiluminescence (Pierce, Life Technologies GmbH, Darmstadt, Germany). Images were captured with the Versa Doc 5000 imaging system (Bio-Rad, München, Germany).

Fluorescence Microscopy

CaF₂ windows with attached cell cultures used previously for the Raman measurements were used for immunofluorescence imaging. After Raman measurements, the formalin-fixed cells were permeabilized with 0.2% Triton X-100 for 5 min at room temperature, washed with PBS, and blocked with 1% bovine serum albumin (BSA) for 30 min. Cells were incubated with the primary mouse monoclonal anti-EGFR (ab30; Abcam, Cambridge, UK) overnight at 4°C, followed by washing with PBS buffer and incubation for 1 h at room temperature with fluorescein-conjugated secondary antibodies (FITC; Jackson ImmunoResearch, West Grove, USA). Excess antibodies were removed by washing several times with PBS buffer. Cells were further incubated with 1,5-bis{[2-(dimethylamino)ethyl]amino}-4,8-dihydroxyanthracene-9,10-dione (DRAQ-5; Cell Signaling Technology, Danvers, USA) or Nile Red for 15 and 30 min, respectively. Then, cells were washed with PBS buffer.

Fluorescence measurements were performed with a confocal microscope (Leica TCS SP5 II) using a Leica HCX PL APO (25×/0.95 NA) water-immersion objective. Nuclear fluorescence was imaged by exciting with the 633 nm laser, whereas the fluorescence of lipid droplets and EGFR was imaged by exciting with the 561 nm laser.

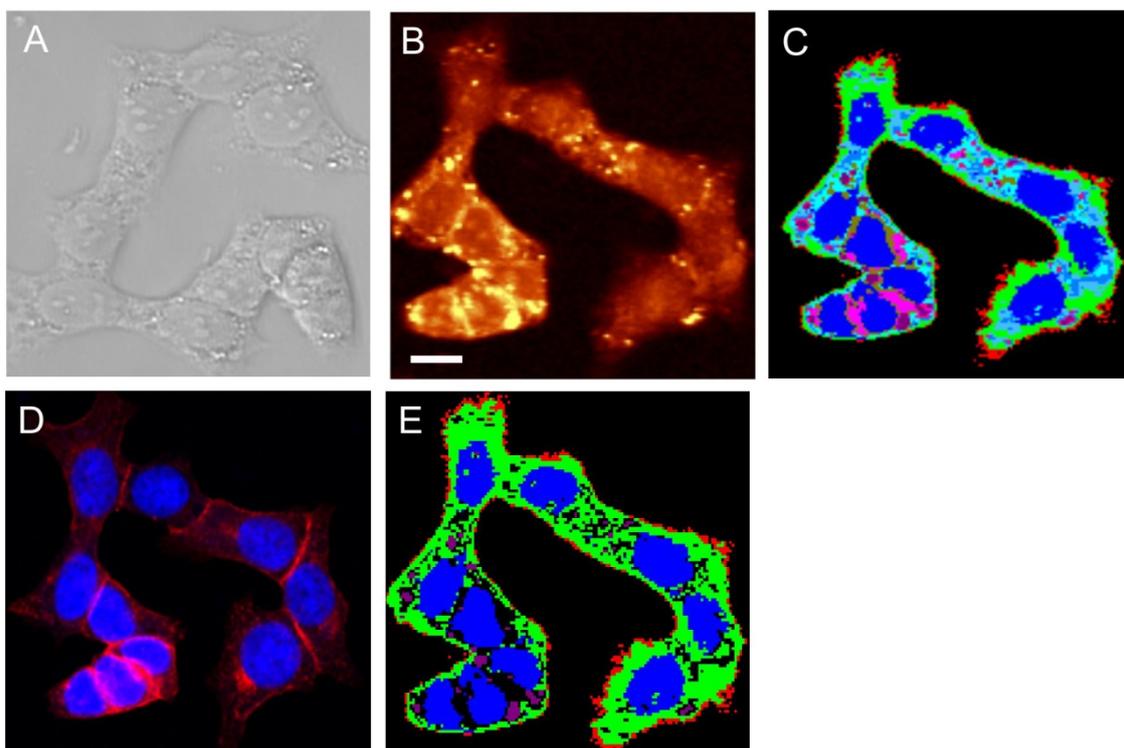


Figure S1. (A) Bright field microscopic image of SW48 cells. (B) Integrated Raman intensities in the C—H stretching region of SW48 cells shown in A. (C) HCA results based on the Raman data shown in B. (D) Fluorescence imaging of the nuclei (blue) and EGFR (red). (E) HCA clusters of membrane (red), cytoplasm (green), nuclei (blue), and lipid droplets (magenta). The scale bar is 10 μm .

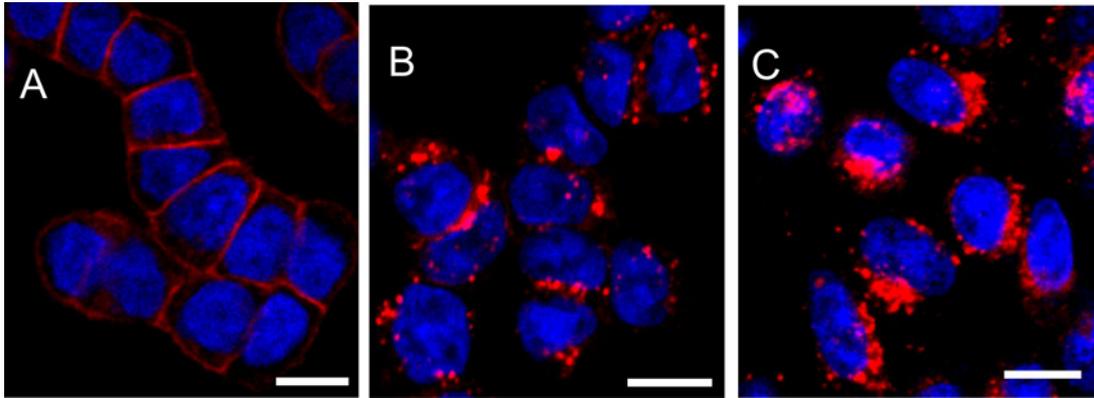


Figure S2. (A) Fluorescence imaging of the nucleus (blue) and EGFR (red) in SW48 cells. Fluorescence imaging in the presence of EGF in (B) SW48 and (C) SW480 cells. Scale bar is 10 μm .

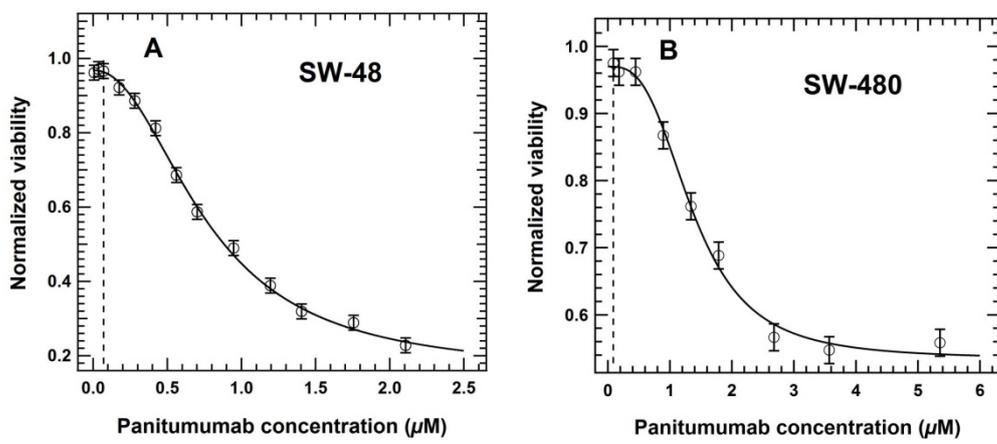


Figure S3. MTT cytotoxicity of SW48 (A) and SW480 (B) cells.

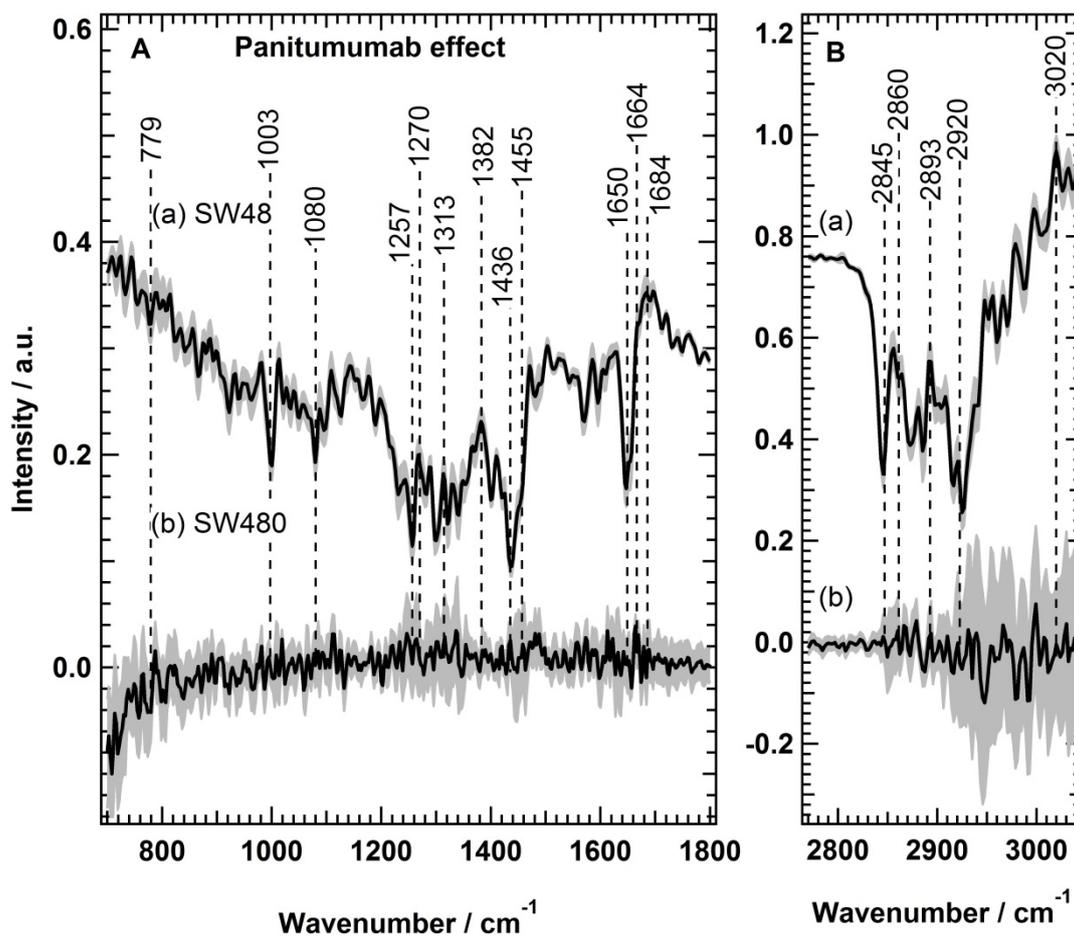


Figure S4. Effect of panitumumab on SW48 (a) and SW480 (b) cells. Raman difference spectra of control *versus* cells treated with panitumumab in the 700–1,800 (A) and 2,770–3,040 cm^{-1} (B) regions reflect the changes in whole cellular components. The shading is the standard deviation.

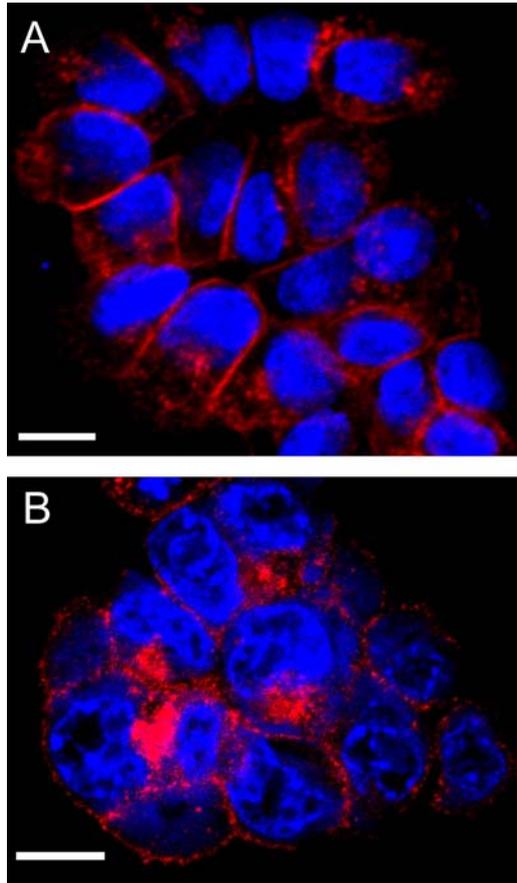


Figure S5. Fluorescence imaging of nucleus (blue) and EGFR (red) of SW48 (A) and SW480 (B) cells treated with panitumumab. The scale bar is 10 μm .

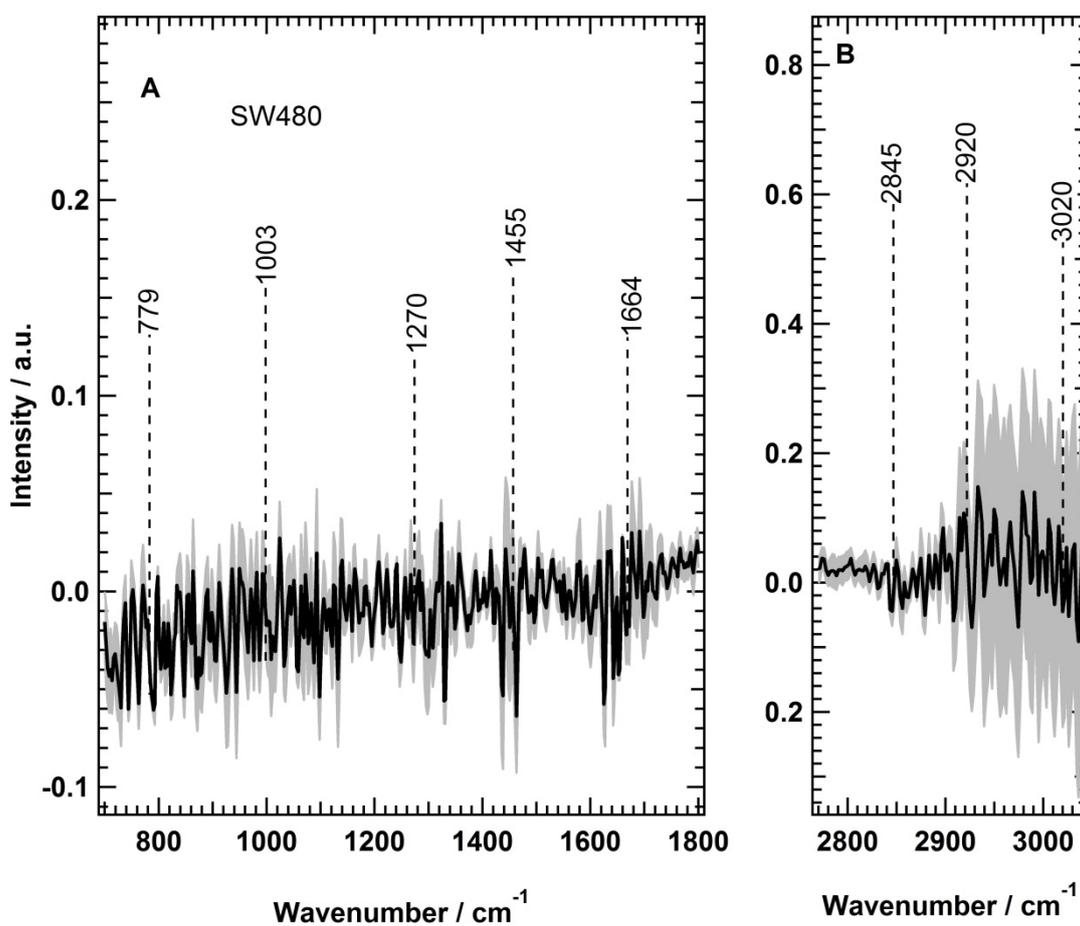


Figure S6. Raman difference spectrum of SW480 cells treated with EGF *versus* cells treated with EGF and panitumumab in the 700–1,800 (A) and 2,770–3,040 cm^{-1} (B) regions. The spectrum shows no significant spectral changes in cells. Shading represents the standard deviation.

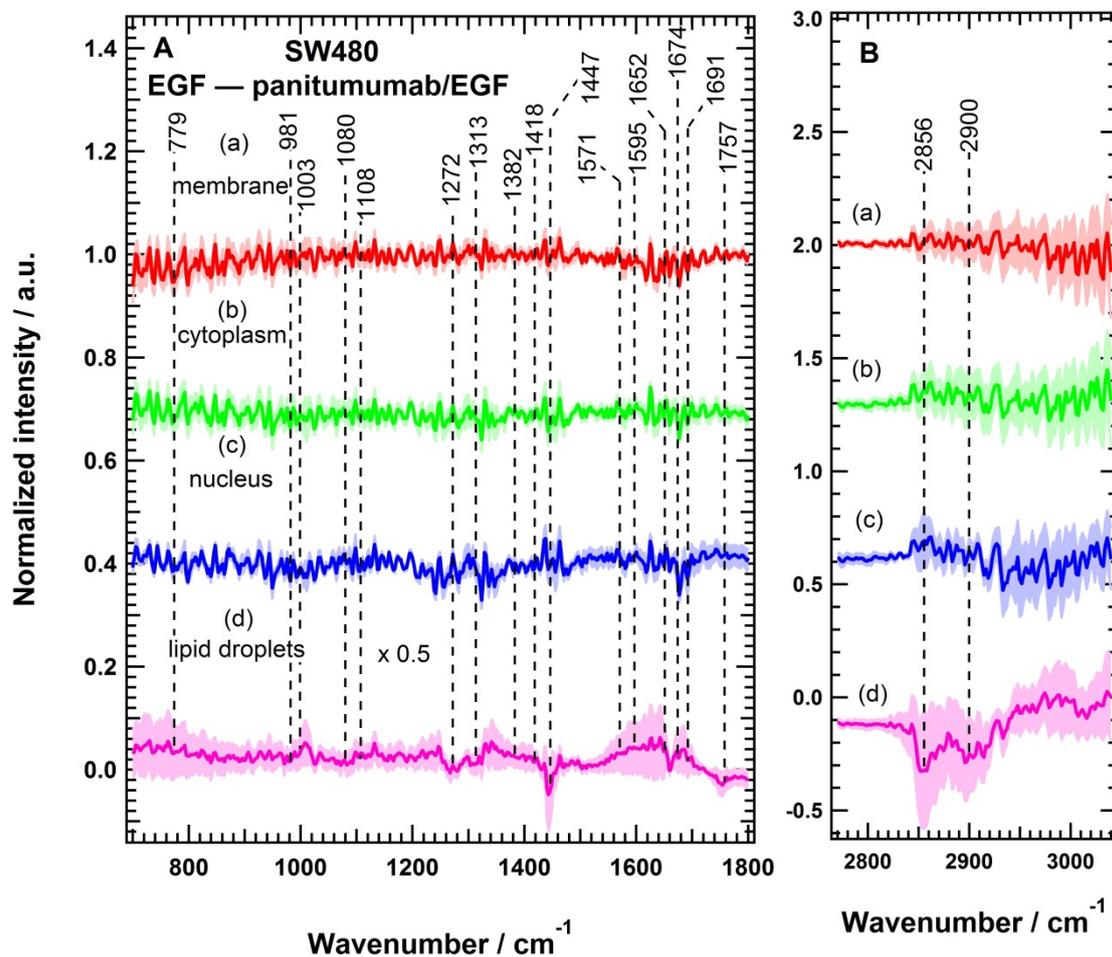


Figure S7. Raman difference spectra of SW480 cells treated with EGF versus cells treated with EGF/panitumumab in the 700–1,800 (A) and 2,770–3,040 cm^{-1} (B) regions for the membrane (a), cytoplasm (b), nucleus (c), and lipid droplets (d). The shading shows the standard deviation.

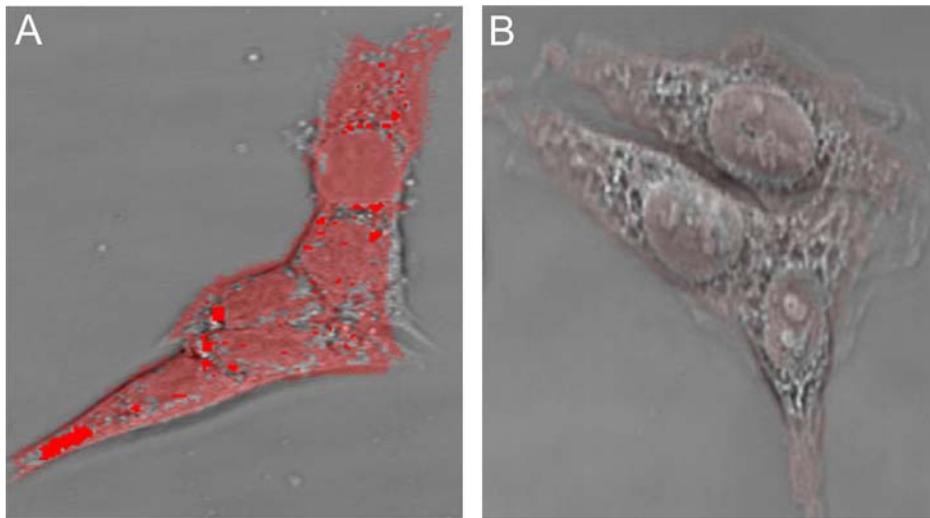


Figure S8. Effect of panitumumab on cells. Overlay of the bright-field image with the HCA image of four clusters (plasma membrane, cytoplasm, nuclei, and lipid droplets) for (A) SW48 and (B) SW480 cells treated with panitumumab. The red color intensity is scaled by the changes observed in the spectra for each cluster. SW48 shows large changes in response to panitumumab treatment, whereas SW480 is essentially unchanged. The cellular changes occurring after panitumumab treatment were quantified by integrating the difference signal exceeding the standard deviation.