Figure EV1. MSOT spectra of liver, kidney, interscapular brown adipose tissue (iBAT), retroperitoneal white adipose tissue (rpWAT), aorta and Sulzer vein (SV) in vivo and main absorbers.

A–C Raw spectra of liver, kidney, iBAT, rpWAT aorta and SV in vivo. Data represent the mean from 8 animals (n = 8).
D Normalized spectra of H$_2$O, Hb, HbO$_2$ and Lipid. Data are provided as reference in ViewMSOT 3.8 software.
Figure EV2. MSOT imaging of lipid phantoms.

A Reconstructed MSOT images (930 nm) of phantoms containing 0–100% lipid. The lipid fraction and the ROI are indicated on each image. The colour bar shows the colour coding of MSOT a.u. from 0 to 2,000 a.u. (bottom to top). Scale bar: 4 mm.

B Absorption of lipid phantoms at 930 nm. Data represent the mean from 3 sections per phantom.

C Linear unmixing result of lipid from the phantoms. Data represent the mean from 3 sections per phantom.
**Figure EV3.** MSOT imaging of liver-lipid phantoms.

A Reconstructed MSOT images (930 nm) of phantoms contains 0–60% lipid. The lipid fraction and the ROI are indicated on each image. The colour bar shows the colour coding of MSOT a.u. from 0 to 7,000 a.u. (bottom to top). Scale bar: 4mm.

B Normalized spectra of phantoms in A. Data represent the mean from 3 sections per phantom.

C Linear unmixing result of lipid in phantoms. Data represent the mean from 3 sections per phantom.

**Figure EV4.** Histology quantification of lipid in liver.

Each dot represents data from one animal (grade 0: \(n = 14\); grade 1: \(n = 11\); grade 2: \(n = 7\); and grade 3: \(n = 9\)). Data represent the mean (± 95% confidence). The unpaired t-test was used to verify the statistical significance. Grade 0 versus grade 1: \(P = 6.80E-11\), grade 1 versus grade 2: \(P = 0.0007\), and grade 2 versus grade 3: \(P = 0.0086\).
Figure EV5. Correlation analysis of different lipid detection methods.
A Correlation between lipid linear unmixing readout and fat quantification by histology. Each dot represents data from one animal (n = 41).
B Correlation between lipid linear unmixing readout and difference analysis readout. Each dot represents data from one animal (n = 41).