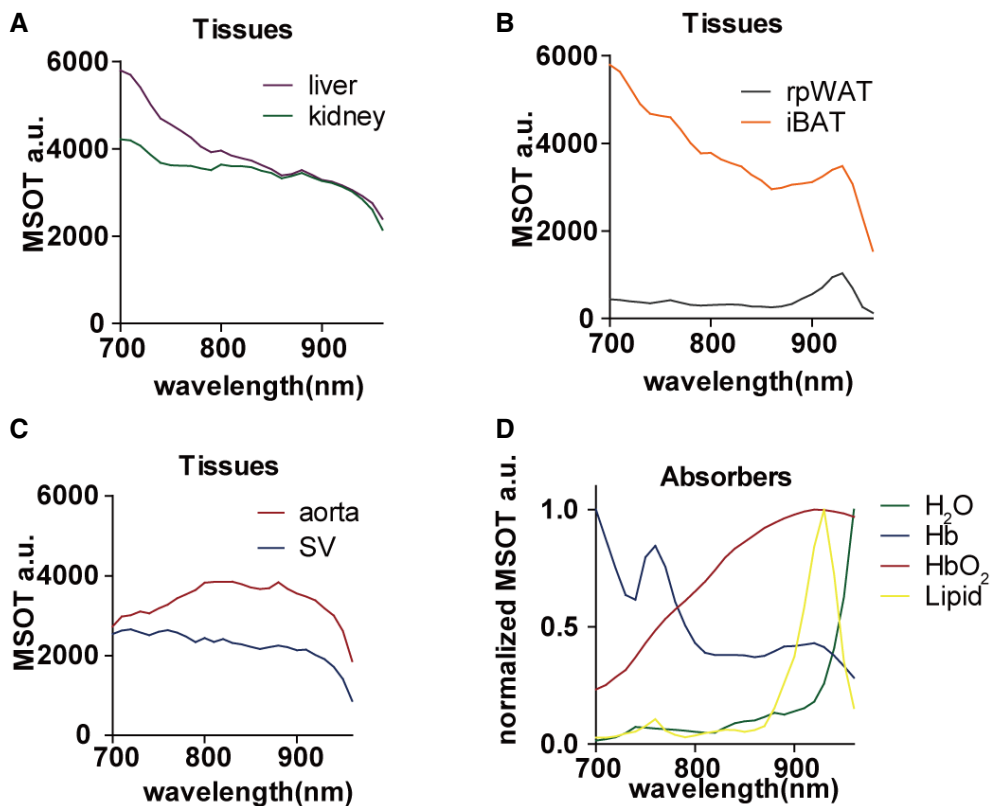


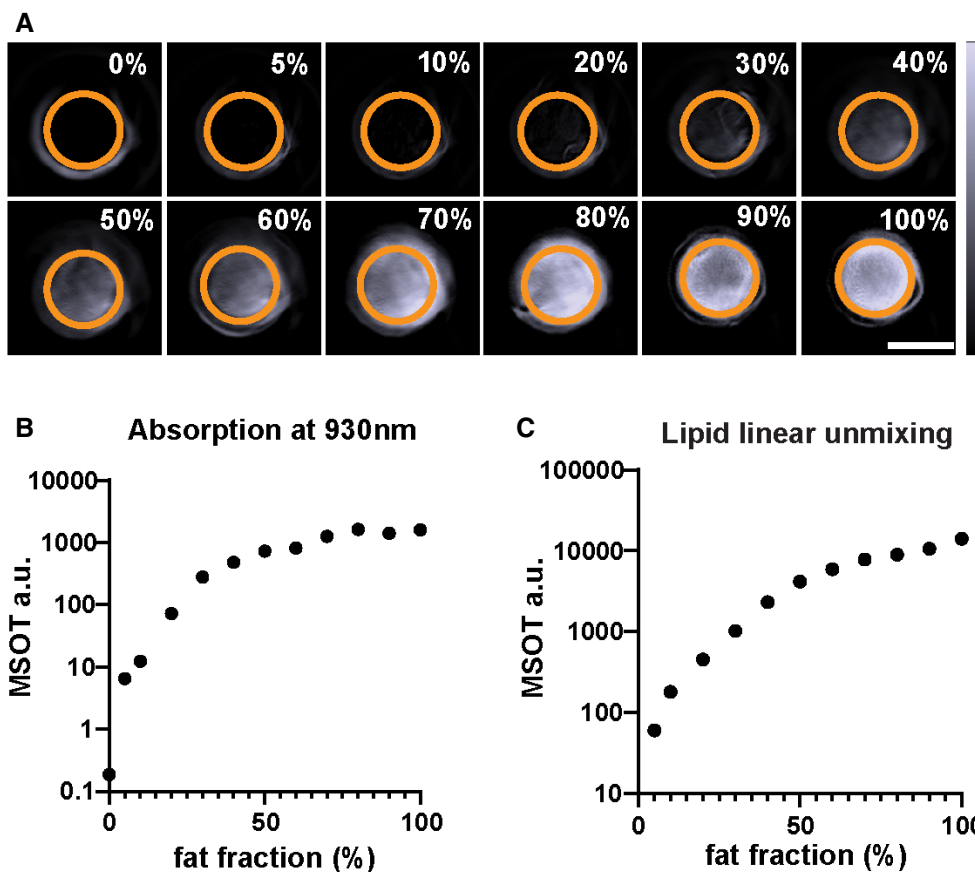
## Expanded View Figures



**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.

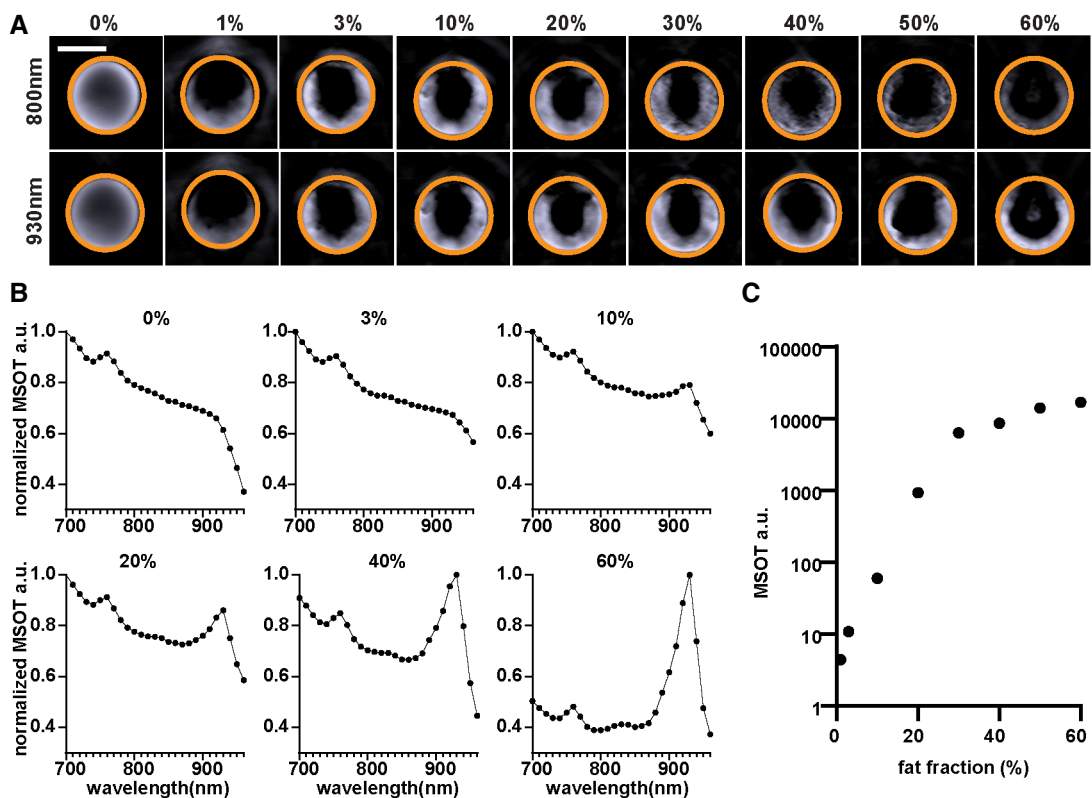
A–C Raw spectra of liver, kidney, iBAT, rpWAT aorta and SV *in vivo*. Data represent the mean from 8 animals ( $n = 8$ ).

D Normalized spectra of H<sub>2</sub>O, Hb, HbO<sub>2</sub> and Lipid. Data are provided as reference in ViewMSOT 3.8 software.



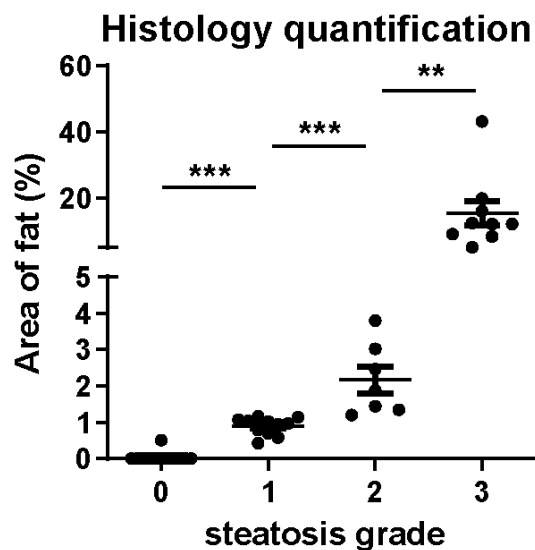
**Figure EV2. MSOT imaging of lipid phantoms.**

- 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.



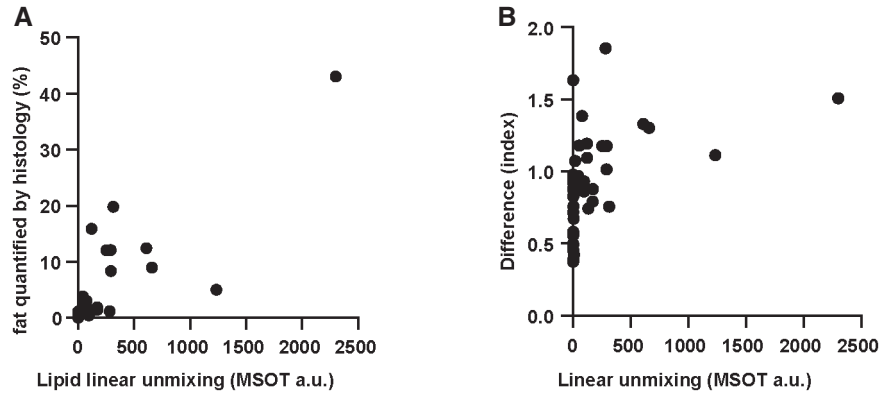
**Figure EV3. MSOT imaging of liver-lipid phantoms.**

- 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 ( $\pm$  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 ( $n = 41$ ).

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