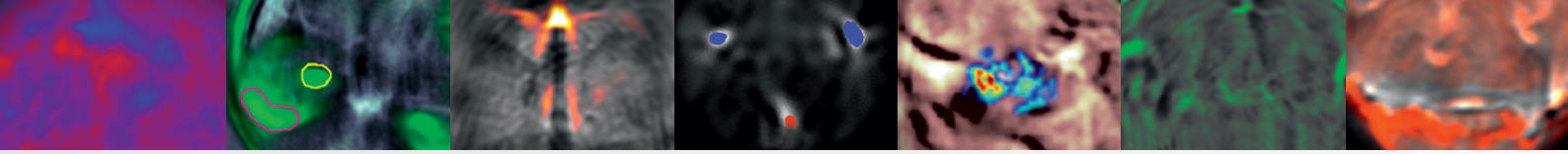
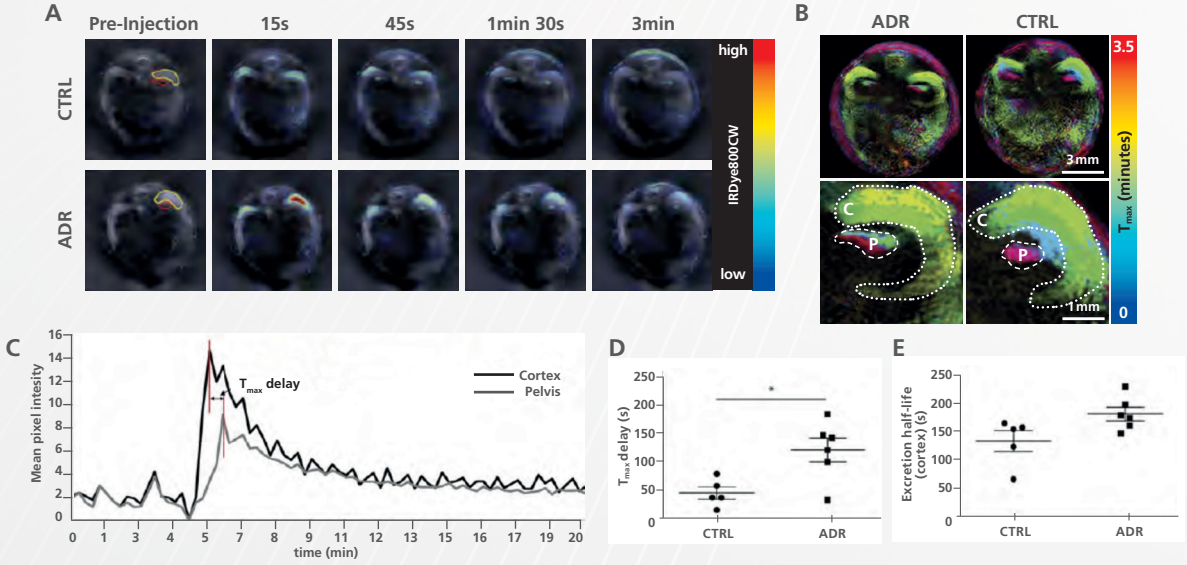


Determining organ function through quantification of MSOT kinetics

Multispectral optoacoustic tomography (MSOT) has been increasingly used for preclinical molecular imaging studies. However, it also holds promise as a functional imaging technique. Akin to the MRI-BOLD imaging sequence, MSOT can quantify oxygenation in tissues as an indicator of neural activity, vascularity and/or tumor activity. Furthermore, MSOT can also be used to assess organ function through the visualization and quantification of contrast agents that are subject to specific clearance mechanisms. This provides an alternative to traditional methods of assessing organ function, which may include invasive blood tests or biopsies, or functional imaging techniques like PET and SPECT, which involve ionizing radiation, or various MRI sequences, which require extensive costs and infrastructure. In preclinical models, MSOT offers high spatial resolution and sub-second temporal resolution with molecular specificity. As such, it offers the ability to non-invasively study organ function and disease repeatedly in the same animals during the development of disease.

Figure 1 demonstrates how MSOT can be used to quantify renal function [1]. IRdye800CW (Li-Cor) undergoes glomerular filtration and is eliminated from circulation by the kidneys. Following systemic injection, the clearance of IRdye800CW can be visualized in cross-sectional images over time (panel 1a), or as a parametric map showing maximum MSOT signal with a temporal color code (panel 2b). Control animals were compared to adriamcycin (ADR)-intoxicated mice with reduced kidney function, with the intoxicated mice showing increased transit times of the contrast agent from the renal cortex to the renal pelvis (panel 1c and 1d).

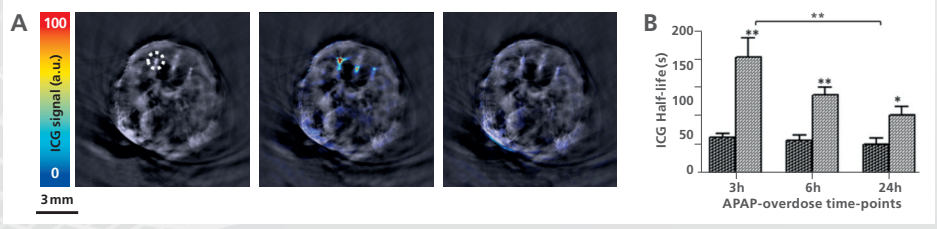
FIGURE 1: Assessment of renal function associated with kidney toxicity



(A) MSOT images of a typical control (top) and ADR-administered (bottom) mouse before and after the administration of IRDye800CW. Region of interests (ROIs) depicted in yellow (cortex) and red (papilla/pelvis region). Times indicated above apply to both control and ADR-treated animal. (B) Temporal color maps of control and ADR-administered mice reveal a delay in IRDye800CW clearance kinetics in the treated mice. (C) Characteristic plot of mean pixel intensity (MSOT arbitrary units) from ROIs drawn around the renal cortex (black) and papilla/pelvis region (grey). (D,E) Graphs showing distribution of TMAX delay (D) and the excretion half-life in the cortex (E) in ADR-administered and control animals. Data points (D,E) represent individual animals (circles = control, n = 5; squares = ADR-administered, n = 6) and lines represent mean ± standard error. Asterisks indicate significance of two-sample t-tests: $p \leq 0.05$ (*).

Figure 2 shows the performance of MSOT with regard to quantifying liver function [2]. Indocyanine green (ICG), which is subject exclusively to hepatobiliary clearance, was injected i.v. into control or acetaminophen (APAP)-intoxicated mice with decreased liver function. The clearance of ICG from the blood (panel 2a) by the liver was quantified as a marker of liver function, showing a slower clearance rate in the intoxicated mice (panel 2b).

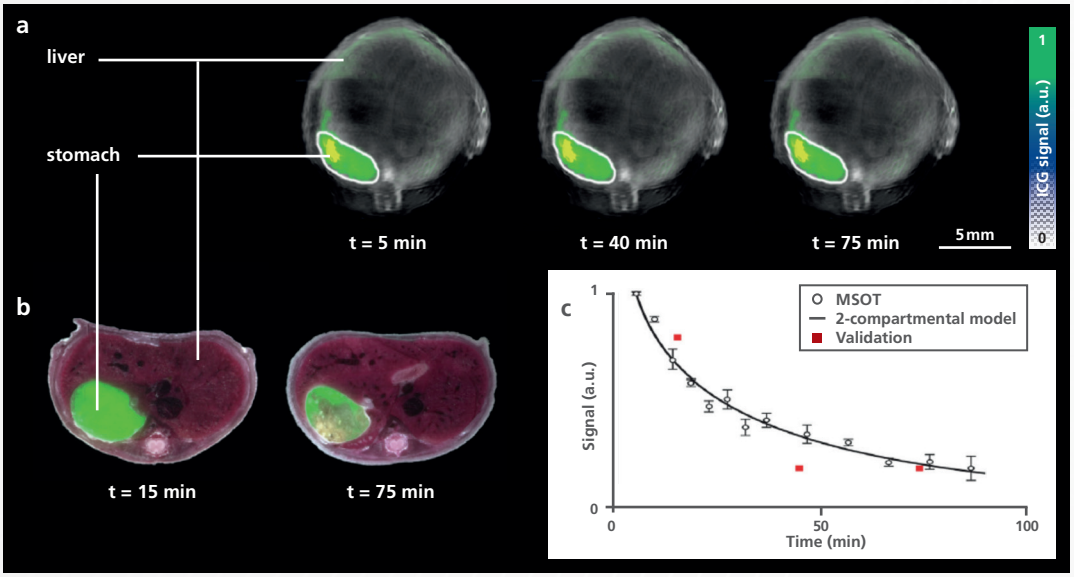
FIGURE 2: Assessment of liver function and toxicity



(A) Region of interest depicted in white (Ischiatic vessels) from the lumbar cross section. (B) ICG half-life significantly increased after APAP-overdose compared to untreated mice at 3, 6 and 24 h. Bars represent mean ± standard error of group of animals (black = control, n = 5; white = APAP (300 mg/kg) overdose, n = 8-10). Asterisks indicate significance of two-sample t-tests: $p \leq 0.05$ (*), $p \leq 0.005$ (**).

A common side effect of medication is gastrointestinal intolerance. Symptoms can include reduced appetite, diarrhea, constipation, gastrointestinal (GI) inflammation, nausea and vomiting. Such effects often have a dramatic impact on compliance with a treatment regimen. Therefore, characterization of GI tolerance is an important step when establishing a novel therapeutic approach. In figure 3, ICG was administered to mice via oral gavage, and whole body MSOT scans over time revealed the clearance of ICG from the stomach, and transit to the GI [3].

FIGURE 3: MSOT imaging of gastric emptying



Panel (a) shows the ICG signal distribution at multiple time points after oral gavage of an ICG-containing solution. MSOT images show a single-wavelength optoacoustic image (grayscale, 900 nm) as an anatomical reference with an overlay of multispectrally resolved ICG signal (green). Panel (b) shows ex vivo sectioning of animals sacrificed at 15 and 75 min post ICG administration, with an RGB image showing reference anatomy and location of the fluorescent ICG shown in green. Panel (c) shows the quantification of MSOT signals from mice (n = 3) (open circles), with modeled data shown as a black line. Red squares indicate fluorescence measured ex vivo in mice (n = 1).

Conclusions

In summary, the study of organ function is paramount for translational research projects. The depth penetration, imaging speed, spatial resolution and molecular specificity of MSOT is well suited to study gastrointestinal, liver and kidney organ function by quantifying the clearance of commercially available contrast agents as surrogate markers of function.

MSOT Imaging Protocol

Acquisition System	Single-Wavelength Image Acquisition/Display Rate	Multispectral Acquisition Wavelengths used	Analysis Method
MSOT inVision 256-TF small animal scanner	10 Hz	700/715/730/760/800/830 and 860 nm	Model-based tomographic image reconstruction; Spectral unmixing by linear regression

References

[1] Scarfe L., Rak-Raszewska A., Geraci S., Darssan D., Sharkey J., Huang J., Burton N.C., Mason D., Ranjzad P., Kenny S., Gretz N., Lévy R., Kevin Park B., García-Fiñana M., Woolf A.S., Murray P., Wilm B., **Measures of kidney function by minimally invasive techniques correlate with histological glomerular damage in SCID mice with adriamycin-induced nephropathy.** Sci Rep. 2015 Sep 2;5:13601.

[2] Courtesy of Dr. Daniel Antoine and Nathalie Brillant, Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, August 2016.

[3] Morscher S., Driessen W.H., Claussen J., Burton N.C., **Semi-quantitative Multispectral Optoacoustic Tomography (MSOT) for volumetric PK imaging of gastric emptying**, Photoacoustics. 2014 Jun 27;2(3):103-10.