Functional tumor imaging using Multispectral Optoacoustic Tomography (MSOT)

Functional measures of tumor vasculature are important for cancer staging and prognosis, including the likelihood of chemo- and radio-resistance. The current clinically-approved imaging modalities for assessing tumor vascular function have major drawbacks, including concerns of long-term toxicity from required contrast agents, as well as poor spatial and temporal resolution. MSOT is an emerging non-invasive and non-toxic imaging modality with capacity for assessing tumor functional metrics in high spatiotemporal resolution and capable of both label-free and contrast-enhanced dynamic functional imaging.

Dynamic functional tumor imaging

Dynamic imaging is useful for determining vascular function in regions of interest. OE-MSOT is a label-free, safe, and clinically translatable method designed to highlight tissue regions responsive to changes in oxygen concentration. The subject is scanned while administered medical air (21% oxygen), then switched to 100% oxygen. Data is spectrally unmixed for oxy- and deoxy-hemoglobin, which are used to calculate SO_2^{MSOT}. Changes in SO_2^{MSOT} signal highlight regions responsive to O_2 modulation. Similarly, DCE-MSOT utilizes injectable contrast such as indocyanine green (ICG) to identify regions with differential perfusion of contrast.

INNOVATIVE
MSOT can differentiate exogenous dyes and endogenous absorbers such as hemoglobin, allowing both dynamic contrast-enhanced and label-free functional characterization of tumors.

NON-INVASIVE
Acquire real-time functional data label-free using oxygen-enhanced MSOT (OE-MSOT) or dynamic contrast enhanced MSOT (DCE-MSOT) with simple injection of a fluorescent dye.

HIGH PERFORMANCE
MSOT functional metrics correlate strongly with endpoint histological markers for vascular maturity, hypoxia, and necrosis.

EASY TO USE
Fully automated data acquisition; user-friendly image reconstruction, quantification, and export, supported by viewMSOT™ software.

IMAGING PROTOCOL

Imaging System	MSOT inVision 256-TF
Repetition Rate	10 Hz
Excitation Wavelength	OE-MSOT: 700, 730, 750, 760, 770, 800, 820, 840, 850 and 880 nm DCE-MSOT: 700, 730, 760, 800, and 850 nm
Processing Methods	Back-projection tomographic image reconstruction; spectral unmixing by linear regression.

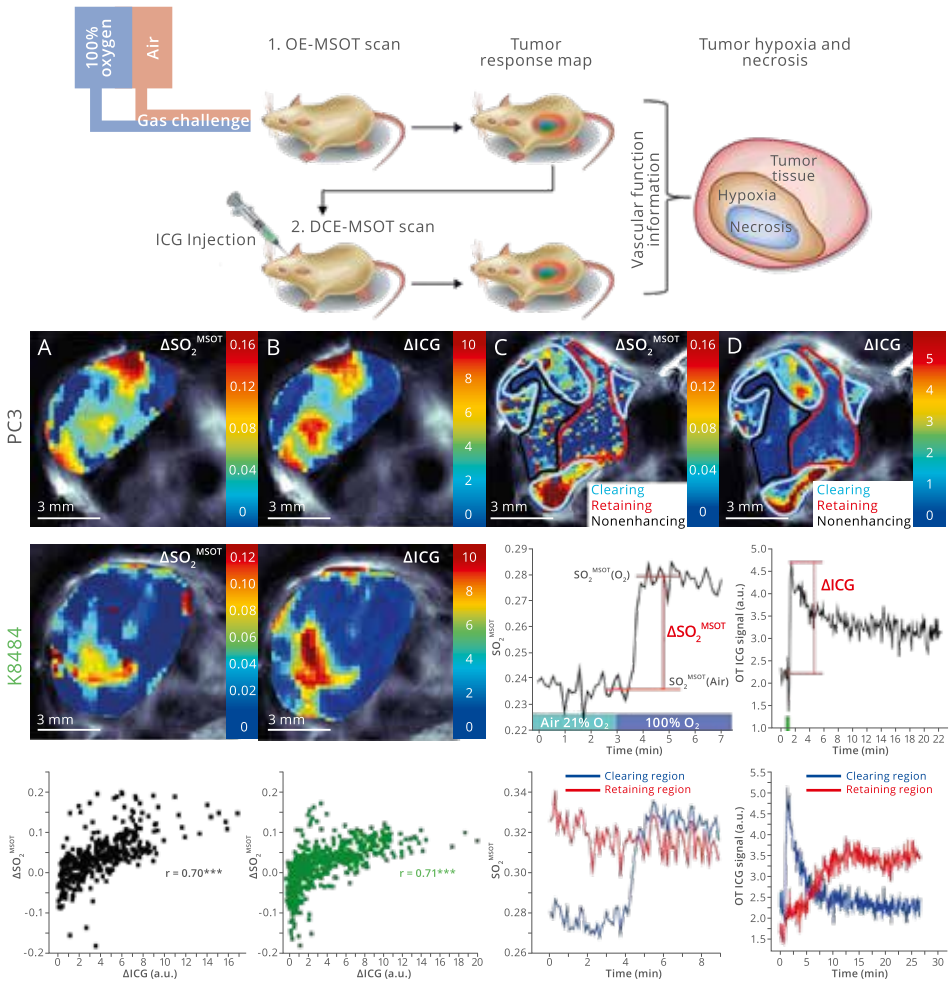

FIGURE 1: Comparison of OE-MSOT and DCE-MSOT

Response maps for two representative tumor types (PC3 and K8484) are shown for (A) ΔSO_2^{MSOT} and (B) ΔICG. Significant spatial correlation between ΔSO_2^{MSOT} and ΔICG is present for each tumor type, indicating that similar vascular characteristics in the tissue are involved for each. Spatially distinct regions segmented to highlight clearing, retaining, and nonenhancing portions of a representative PC3 tumor show high similarity with both (C) ΔSO_2^{MSOT} and (D) ΔICG response maps. SO_2^{MSOT} and ICG kinetic curves are shown to illustrate signal change over time for the entire tumor region. Kinetic plots of subdivided clearing and retaining regions are also shown.

Ex vivo validation

While the spatial maps of OE-MSOT and DCE-MSOT are strongly correlated, each shows distinct relationships to underlying tumor physiology. In figure 2, the spatial relationships of OE-MSOT and DCE-MSOT are correlated to clinically relevant biomarkers of vascular maturity (ASMA), hypoxia (CAIX), and necrosis (H&E). These results indicate that OE-MSOT signal is driven predominately by tumor hypoxia and necrosis, whereas DCE-MSOT signal most strongly reflects vascular maturity as it relates to perfusion.

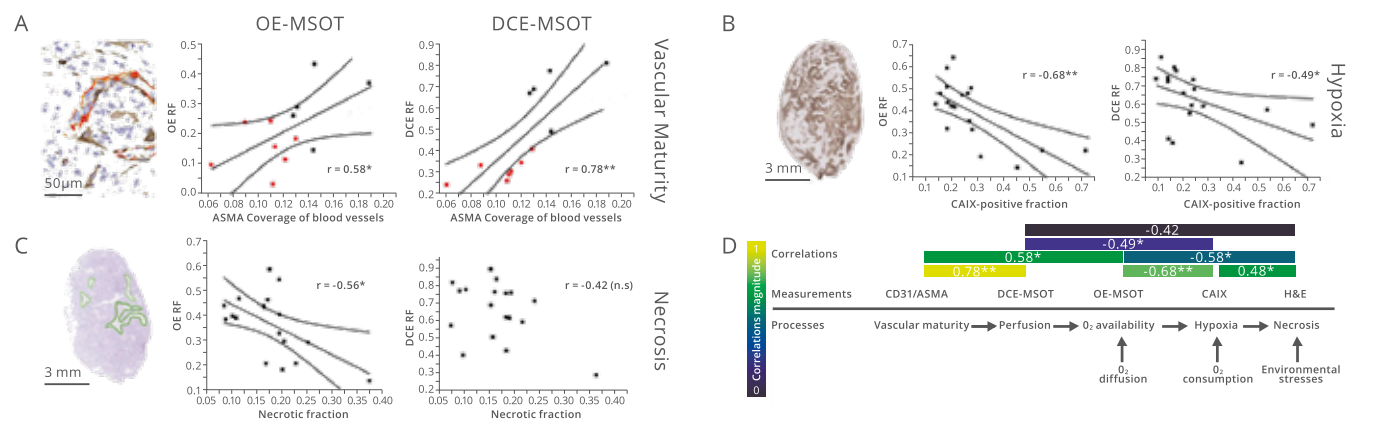

FIGURE 2: Functional assessment of tumor vasculature and oxygenation using OE-MSOT

Spatial correlations of responding fraction (RF) pixels from OE-MSOT and DCE-MSOT with histological stains (A) ASMA (B) CAIX, and (C) H&E. ASMA-positive pixels highlight the fraction of mature vessels, which correlate significantly with responding fractions of DCE-MSOT (r=0.78**) and OE-MSOT to a lesser extent (r=0.58*). CAIX-positive fractions illustrate regions of hypoxia, which correlate significantly with OE-MSOT (r=-0.68**) and less significantly with DCE-MSOT (r=-0.49*). Necrotic fractions determined by H&E staining correlate significantly with OE-MSOT (r=-0.56*) but show no significant correlation to DCE-MSOT. (D) Summary of correlations and their relationships to tumor physiological processes.

Therapeutic response

Changes in vascular function are indicative of response to therapeutic intervention. Vascular disrupting agents such as CA4P target the vascular structure of tumors by disrupting the VE-cadherin signalling, ultimately blocking the flow of blood to the tumor. Dynamic imaging with OE-MSOT and DCE-MSOT 48 hours prior to and four hours following treatment with CA4P demonstrate the loss of vascular function in the tumor. Following treatment, a substantial decrease in the responding fraction of pixels in OE-MSOT and DCE-MSOT is apparent, indicating a significant loss of oxygen availability and perfusion within the tumor, respectively.

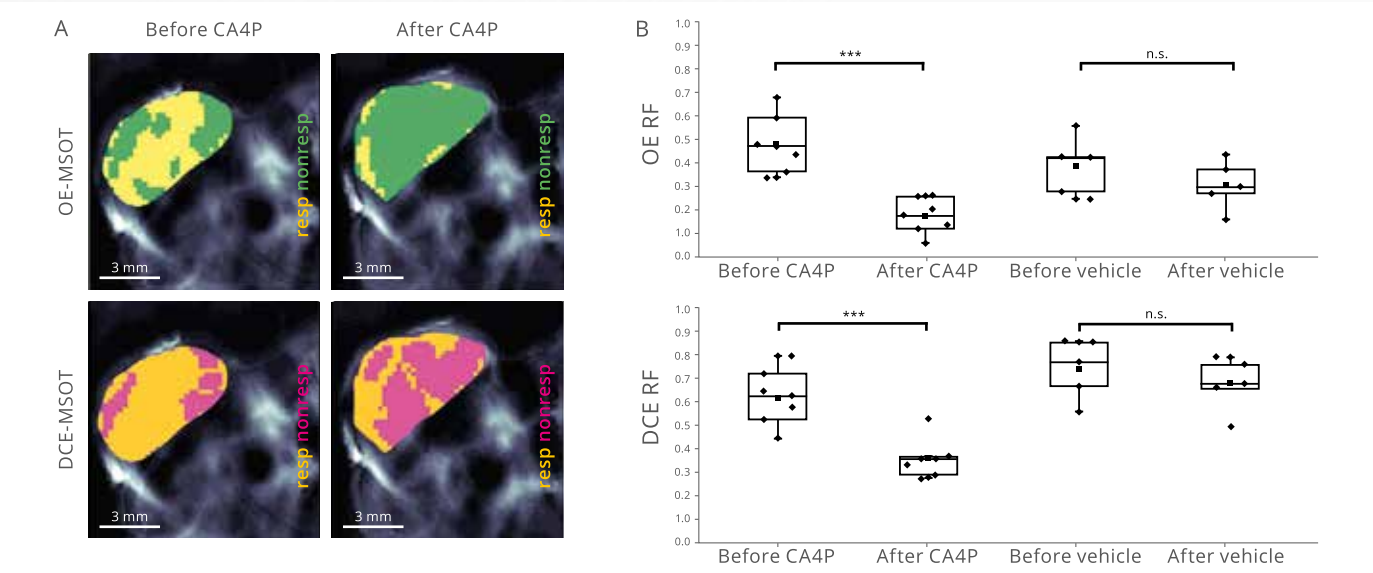

FIGURE 3: Tumor vascular response to therapy

(A) Responding fractions of pixels from OE-MSOT and DCE-MSOT before (left) and after (right) treatment with vascular disruptor CA4P. OE-MSOT and DCE-MSOT each reveal a dramatic decrease in responding pixels following CA4P treatment, indicating that these methods can be used to assess treatment efficacy as it relates to tumor vasculature. (B) Quantification of OE-RF and DCE-RF before and after treatment with CA4P. No significant change was observed in vehicle groups, while a highly significant decrease in responding pixel fractions was observed for both OE-MSOT and DCE-MSOT.

[1] Tomaszewski et al., **Oxygen-Enhanced and Dynamic Contrast-Enhanced Optoacoustic Tomography Provide Surrogate Biomarkers of Tumor Vascular Function, Hypoxia, and Necrosis**, Cancer Res. 2018 Oct 15;78(20):5980-5991.