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Listening to Molecules

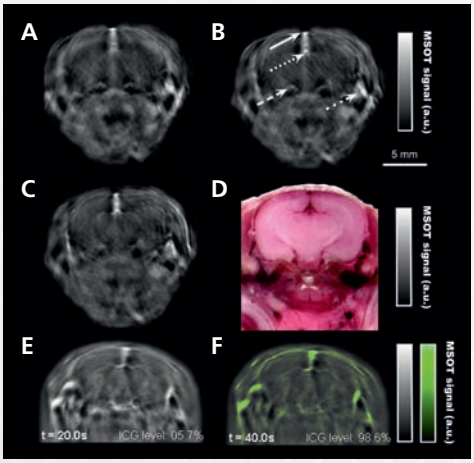
Multispectral Optoacoustic Tomography (MSOT) Brain Imaging and Characterization of Glioblastoma

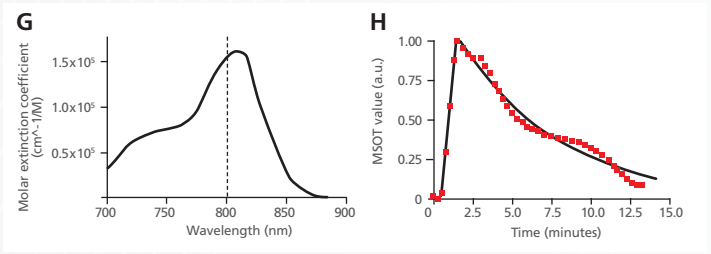
Neurological research depends strongly on imaging for assessing both brain function and aspects of disease *in vivo*. Multispectral Optoacoustic Tomography (MSOT) is a novel technology for high-resolution structural, functional and molecular imaging, through intact skin and skull.

By being able to detect absorbing molecules centimeters deep in tissue, MSOT represents an ideal modality for small animal brain imaging. MSOT offers unprecedented performance in cross-sectional brain imaging of anatomical and physiological parameters in mice. MSOT can quantify the kinetics of perfusion and clearance of optical imaging agents such as Indocyanine Green (ICG) (Figure 1). Further, changes in brain blood oxygenation can be determined by MSOT (Figure 2), for example to resolve ischemic areas of the brain.

FIGURE 1: *In vivo* MSOT brain imaging and pharmacokinetic modeling.

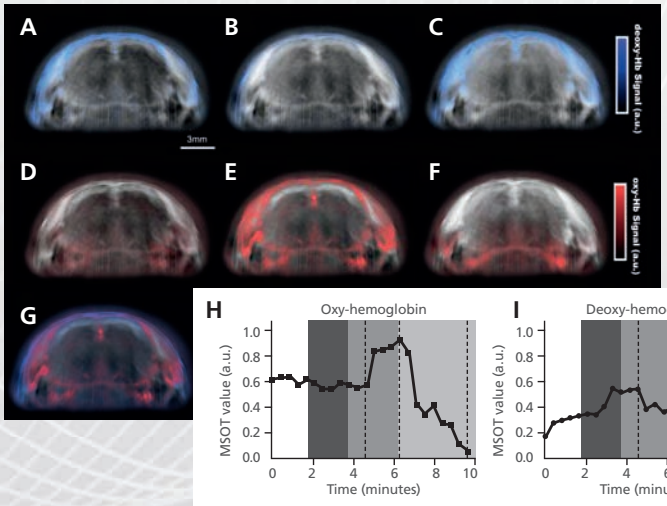
Single-wavelength (800nm) anatomical optoacoustic images of an intact brain from a living mouse (panels A-C, at Bregma 0.26mm, -0.94mm and -2.18mm, respectively). In panel B, brain structures such as the superior sagittal sinus (solid arrow), the third ventricle (dotted arrow), the posterior cerebral arteries (arrow with long dashes), and the superficial temporal arteries (arrow with short dashes) can be seen.





Panel D shows reference anatomy from a frozen sectioned mouse at Bregma -2.18mm. In panels E-H, 50nmoles of ICG were injected into the tail vein of a CD1 nude mouse and the uptake of ICG in the vasculature of the brain was monitored in real time by MSOT. Panels E and F show a single-wavelength optoacoustic anatomical image (grayscale) five seconds prior to injection (panel E) and forty seconds after injection (panel F) with the increase in optoacoustic amplitude shown in green. Panel G shows the absorption spectrum of ICG with the wavelength used in the acquisition of experimental data, 800nm, shown as a dashed line. Panel H shows the quantification of ICG signal in the superior sagittal sinus during the 14 minutes following injection, with experimental data in red and modeled data in black.

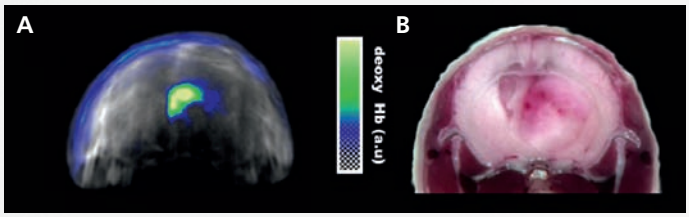
FIGURE 2: Brain blood oxygenation following carbon dioxide challenge.



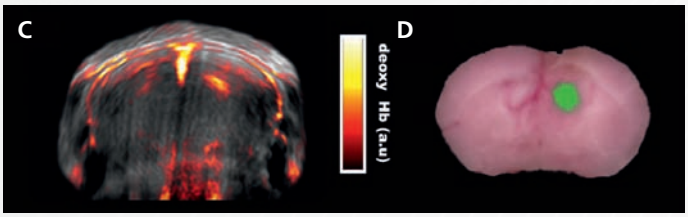
While continuously acquiring multispectral measurements, the air mixture that the animal inspired was successively changed. The graphs in H and I are color-coded to show the temporal changes in inspired air (white, normal air; dark gray, 10% carbon dioxide; intermediate gray, 100% oxygen; and, light gray, 100% carbon dioxide). Figures A, B and C show deoxy-hemoglobin pseudocolor images from a single animal at 4.6, 6.2 and 9.6 minutes, respectively, with increasing deoxygenation depicted in blue. Figures D, E and F show corresponding oxy-hemoglobin in red at the same time points. Panel G shows a combination of oxy- and deoxy-hemoglobin signals at 6.2 minutes (corresponding to panels B and E). Panels H and I plot the corresponding oxy- and deoxy-hemoglobin signals, respectively, throughout the experiment.

Multiparameter, multispectral imaging enables the visualization of the growth of U-87 MG tumor cells injected into the brain (Figure 3). In addition, optical agents injected into the brain can be visualized (Figure 3E). MSOT allows time-dependent detection and quantification of brain parameters that are unavailable using other imaging methods without invasive procedures.

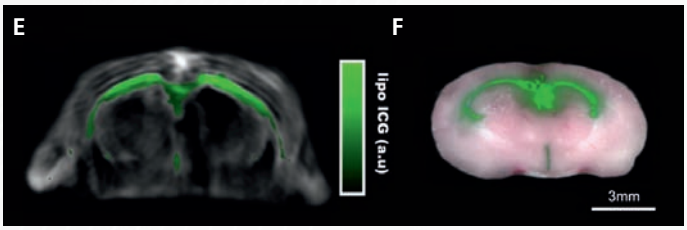
FIGURE 3: Visualization of glioblastoma and liposomal ICG distribution in brain.



Panel A shows the spectrally unmixed deoxy-hemoglobin pseudocolor overlay on a 800nm single-wavelength MSOT image from an animal 34 days post implantation with U87 glioblastoma cells with a corresponding *ex vivo* cryosection in B.



Panel C shows a deoxy-hemoglobin image 16 days following implantation following a 10% carbon dioxide challenge with corresponding *ex vivo* fluorescence image from IntegriSense 750 showing tumor size and location (panel D).



Panels E and F show a mouse injected intraventricularly with ICG encapsulated into liposomes. Panel E shows a 900nm single-wavelength MSOT image (grayscale) with an overlay (green) of the spectrally resolved liposomal ICG signal. Panel F shows the equivalent cryoslice with an overlay of the fluorescence from the injected particles.

The whole-body small animal MSOT scanners generate cross-sectional tomographic images with an in-plane spatial resolution of 150µm. The acquisition and reconstruction time is <100ms per single-wavelength image, thus generating multispectral cross-sectional data in less than 1 second. This high spatial and temporal resolution allows the imaging of blood vessels and regions of interest in the brain with the possibility to probe the functional and molecular aspects of neurological disease.

MSOT Imaging Protocol

Acquisition System	Single-Wavelength Image Acquisition/Display Rate	Multispectral Acquisition Wavelengths Used	Analysis Method
MSOT small animal scanner	10Hz	**ICG and Oxy/Deoxy-Hemoglobin:** 700/730/760/800/850nm	Model-based tomographic image reconstruction; guided & blind spectral unmixing

References:

Burton NC, Patel M, Morscher S, Driessen W, Claussen J, Beziere N, Jetzfellner T, Taruttis A, Razansky D, Bednar B, Ntziachristos V, **"Multispectral Optoacoustic Tomography (MSOT) Brain Imaging and Characterization of Glioblastoma"**, Neuroimage 2012, http://dx.doi.org/10.1016/j.neuroimage.2012.09.053.