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Listening to Molecules

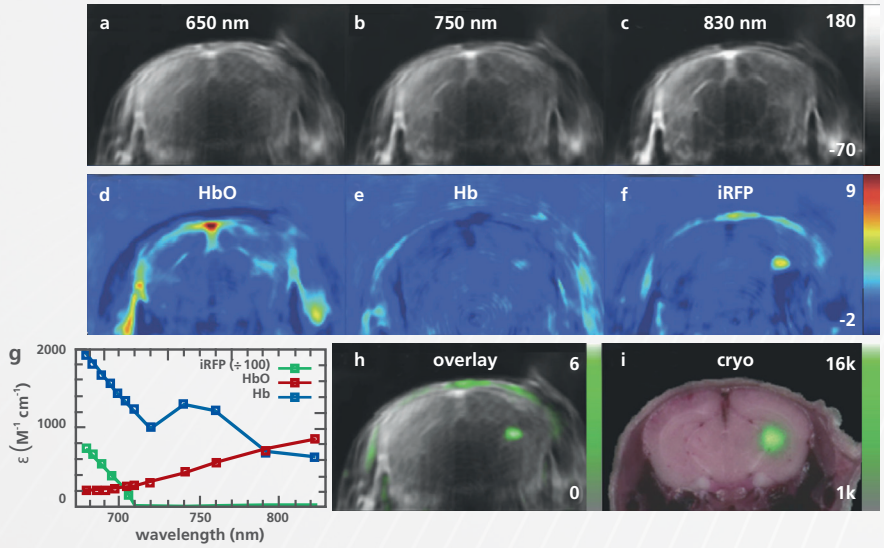


# Visualization of tumor characteristics with targeted ligands using MSOT

**Molecular imaging allows the non-invasive visualization of cellular function and the characterization of pathological processes. Multiple imaging modalities have evolved over time, each with their own benefits and limitations. Multispectral optoacoustic tomography (MSOT) offers molecular specificity with high resolution at 2-3 cm depth in tissue, thus enabling preclinical tumor targeting studies that can be used to assess tumor growth and therapy response. The examples below demonstrate the potential of tumor targeting with MSOT.**

Figure 1 demonstrates how MSOT can visualize signals localized in tumors; for example, in the case of the use of a genetic reporter such as iRFP [1]. Single wavelength images (panels 1a-c) and oxyhemoglobin (panel 1d) do not highlight the tumor; however, deoxy Hb (panel 1e) shows limited tumor specificity. Unmixing for iRFP (panel 1f) shows excellent specificity, as the size, shape and location of the glioblastoma tumor very closely matches validation studies derived from ex vivo sectioning with fluorescence detection (panel 1i).

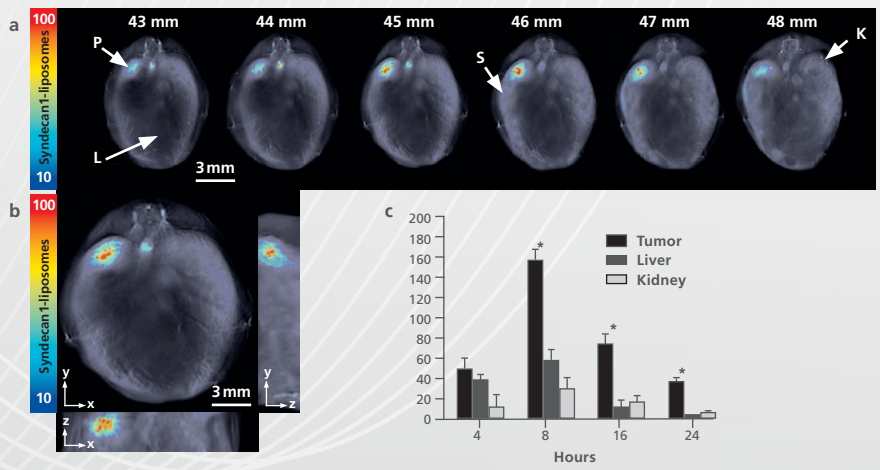
## FIGURE 1: Genetic reporters demonstrate the potential for tumor targeting with MSOT




MSOT imaging and spectral unmixing of a U87 iRFP brain tumor 25 days post implantation. (a–c) Representative reconstructions of acoustic signals at the mouse head at 695, 750, and 830 nm. (d–f) The three component images corresponding to the most prominent absorbers: oxy-, deoxy-hemoglobin, and iRFP, and (g) their corresponding absorption spectra. (h) FP component overlay on the single wavelength "anatomical" image. (i) Comparative fluorescence cryo-section image.

Figure 2 shows an example of tumor targeting using a peptide ligand specific for syndecan-1, which is a protein associated with avb3 integrin expression as well as cellular proliferation [2]. Two-dimensional cross-sectional images (panel 2a) show probe accumulation throughout the tumor. This stack of images can also be rendered as a 3D volume and displayed as orthogonal maximum intensity projections (panel 2b). Biodistribution and tumor targeting over a 24 hour period following injection can also be quantified (panel 2c).

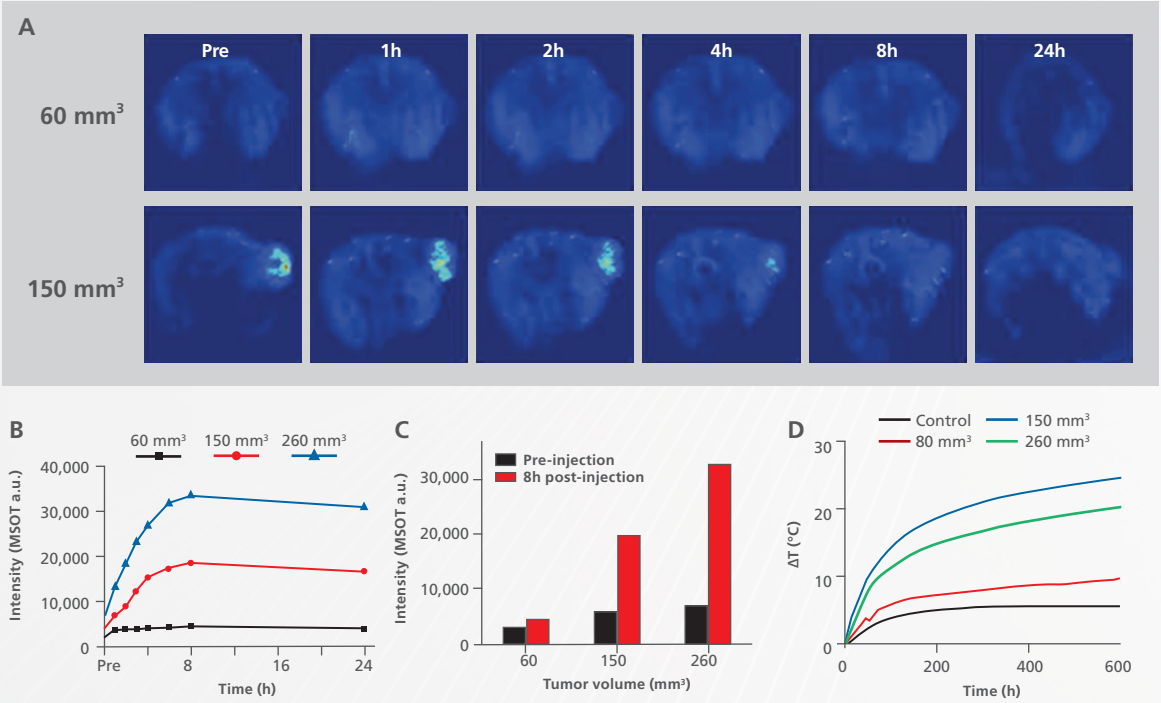
## FIGURE 2: Orthotopic pancreas tumor targeting with a peptide-based ligand, Syndecan1




(a) Serial slice images of liposomal accumulation taken from 43 to 48 mm (abdomen). The highest signal intensity of the liposomes was at 45–46 mm. Organs are identified: P pancreas tumor, L liver, S spleen, K kidney. (b) Orthogonal views of the pancreatic tumor and liposomal accumulation through different anatomical planes. (c) ROI analysis of liposome signal in various locations over time measured in MSOT a.u. Bar height represents the median value and error bars represent the standard deviation throughout the organ. Peak liposomal accumulation occurred at 8 h post-injection. Significantly more liposomes accumulated in the tumor versus off-target organs ($p < 0.05$).

Figure 3 shows pH-sensitive nanoparticles used to target tumors for subsequent phototherapy studies [3]. Targeting efficiency for small and medium sized tumors is shown in panel 3a, while probe accumulation can also be quantified (panel 3b). The direct relationship between probe accumulation as assessed by MSOT (panel 3c) and increases in temperature in the tumor following laser illumination (panel 3d) demonstrates that MSOT signals can be a predictive marker for successful phototherapy.

## FIGURE 3: Targeting with pH-sensitive nanoparticles




*In vivo* photoacoustic imaging and photothermal effect of Fe(III)–gallic acid nanoparticles. (a) Photoacoustic images of mice bearing different sized tumors before injection and at different time points post-injection with Fe(III)–gallic acid nanoparticles. (b) Photoacoustic signal variations of tumor sites in part (a) as a function of post-injection time. (c) Photoacoustic signals of different sized tumors before injection and 8 h post-injection of Fe(III)–gallic acid nanoparticles. (d) Tumor temperature changes in mice bearing different sized tumors during laser irradiation.

## Conclusions

In summary, the above examples demonstrate the promise of molecular imaging with MSOT. Spectral unmixing allows the production of images specific for a molecule of interest, and these molecular signals can be used to determine the size, shape and location tumors, as well as to predict therapy response with photo-sensitive contrast agents.

## 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] Deliolanis NC, Ale A, Morscher S, Burton NC, Schaefer K, Radrich K, Razansky D, Ntziachristos V. **Deep-tissue reporter-gene imaging with fluorescence and optoacoustic tomography: a performance overview.** Mol Imaging Biol. 2014 Oct;16(5):652-60.

[2] Yin W, Kimbrough CW, Gomez-Gutierrez JG, Burns CT, Chuong P, Grizzle WE, McNally LR. **Tumor specific liposomes improve detection of pancreatic adenocarcinoma *in vivo* using optoacoustic tomography**, J Nanobiotechnology. 2015 Dec 1;13:90.

[3] Zeng J, Cheng M, Wang Y, Wen L, Chen L, Li Z, Wu Y, Gao M, Chai Z. **pH-Responsive Fe(III)-Gallic Acid Nanoparticles for *In Vivo* Photoacoustic-Imaging-Guided Photothermal Therapy,** Adv Healthc Mater. 2016 Apr 6;5(7):772-80.