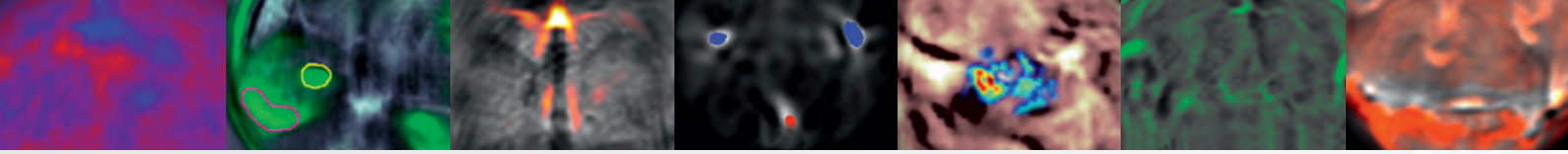


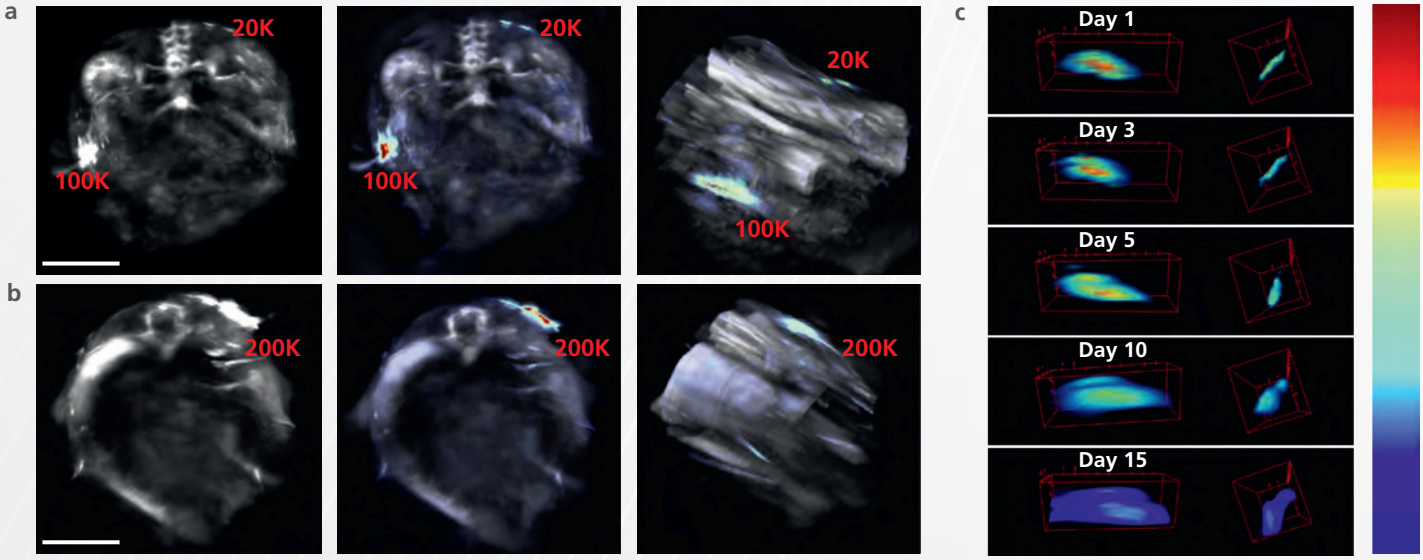


# Tracking Labeled Cells with Multispectral Optoacoustic Tomography (MSOT)

**Tumor development is mainly driven by abnormal growth of cancer cells. Being able to gain insights into the recruitment and persistence of cells into tumor tissue is important for the understanding of tumor heterogeneity. MSOT technology can be leveraged for tracking the tumor distribution of cells loaded with NIR-absorbing agents or genetic reporters such as iRFP.**

Comenge et al. [1] used MSOT to image transplanted murine mesenchymal stem cells (mMSCs) labeled with gold nanorods (GNRs). A silica coating (Si35) of the GNRs was prepared to minimize aggregation after cellular uptake. The labeled cells were injected s.c. at different cell densities. Animals were imaged by MSOT up to 15 days following injection. Figure 1 shows MSOT imaging of labeled cell clusters. In (a) (b), the left column shows a single wavelength maximum intensity projection in the axial plane of the regions of interest. The high spatial resolution of MSOT is observed here, allowing visualization of small vessels (e.g., renal artery and renal vein). The middle column shows the same regions after spectral unmixing, enabling high sensitivity detection of GNR-Si35-labeled cells. Volumetric views of the regions of interest are shown in the right column. As shown in (c), thanks to the high spatial resolution of MSOT, the small volume changes of a cell cluster can be monitored in 3D along the course of the experiment.

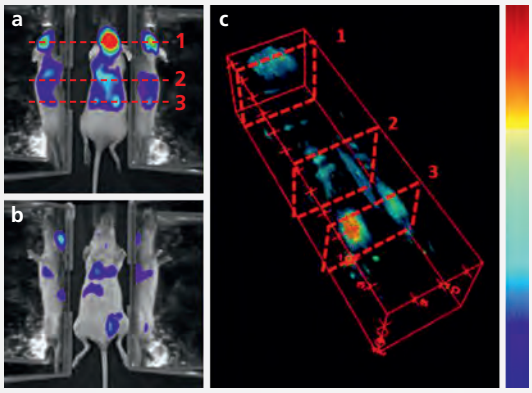
**FIGURE 1: MSOT imaging of GNR-labeled cell clusters**




(a and b) MSOT imaging of the cell clusters 3 days after injection of $2 \times 10^4$ (a), $1 \times 10^5$ (a), and $2 \times 10^5$ (b) GNR-Si35-labeled cells. Scale bars are 5 mm. Color scale range is 0 to $1.1 \times 10^5$ MSOT intensity units (a.u.). Left image is a 680nm MSOT image, the middle image shows an overlay of iRFP-specific signal, and the right image is a 3D rendering of the abdomen of the mouse. (c) Monitoring the growth of the $2 \times 10^5$ cell cluster. Scale of the box is in mm.

In another example, Comenge et al. [2] demonstrated a multimodal imaging approach, which utilizes a combination of GNRs and reporter genes to track cells immediately after systemic injection and longitudinally during tumor development. GNRs/ iRFP720-labeled mMSCs were injected into the left ventricle of the heart. The initial distribution of the injected cells was visualized by MSOT GNR-860 signal, as shown in Figure 2.

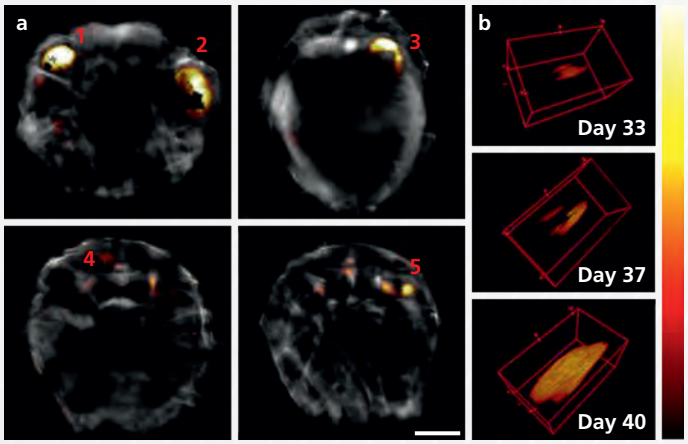
## FIGURE 2: Initial biodistribution of GNR-860 labeled cells after intracardiac administration




Bioluminescence imaging in dorsal (top panel, a) and ventral (bottom panel, b) positions after cell administration. Mirrors were placed on the side of the mouse to provide a lateral view. (c) MSOT imaging of the same animal: Volumetric MSOT imaging for the whole animal showed the correlation between GNR-860 signal with bioluminescence imaging. 1, 2 and 3 refer to comparable areas of the mouse imaged by bioluminescence and MSOT. Color scale range is 0.9 to 21 MSOT intensity units (a.u.).

At later timepoints, the iRFP720 signal intensity was visible while the GNR signal was no longer detectable. Figure 3 (a) shows the presence of multiple tumors 40 days after injection of the cells. A late-developing (days 33-40) tumor in the left shoulder was visualized, demonstrating the capabilities of MSOT to detect iRFP720 with high spatial resolution in 3D, as shown in (b).

## FIGURE 3: Longitudinal tumor monitoring in a mouse that was injected with GNR-860/iRFP720 labeled cells




(a) MSOT imaging of tumors 40 days after systemic injection of the cells. The mouse developed tumors in both shoulders (1, 2), in the right dorsal kidney/adrenal gland region (3), towards the liver (4), and in several positions of the hip region (5) (b). Growth of the tumor on the left shoulder was monitored from day 33 to day 40 and is shown in 3D reconstruction. Color scale range is 1.2 to 14 MSOT intensity units (a.u.).

In summary, MSOT tracks labeled cells with high sensitivity. The 150µm in-plane spatial resolution of MSOT allows the detection of small volume changes of labeled cell clusters or tumors. Longitudinal tracking of labeled cells by MSOT helps understanding the fate of exogenous cells.

## 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 | 20 wavelengths between 690 and 910 nm (for GNR-Si35-labeled cells)<br>32 wavelengths between 660 and 1100 nm (for GNR-860/iRFP720 labeled cells) | model-based tomographic image reconstruction<br>linear regression spectral unmixing (for GNR-Si35-labeled cells)<br>guided ICA spectral unmixing (for GNR-860/iRFP720 labeled cells) |

## References

[1] Comenge J et al.,
**Coupling between Gold Nanorods Improves the Sensitivity of Photoacoustic Detection of Labeled Stem Cells *in Vivo,***
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[2] Comenge J et al.,
**Multimodal cell tracking from systemic administration to tumour growth by combining gold nanorods and reporter genes,**
Elife. 2018 Jun 27;7.