Non-invasive assessment of melanoma metastasis to sentinel lymph nodes using Multispectral Optoacoustic Tomography

Malignant melanoma is the fastest-growing form of cancer and has a 5-year survival rate below 20% in advanced stages. Sentinel lymph node (SLN) metastasis is the most important prognostic indicator for overall survival. SLN biopsy (SLNB) requires surgical excision of SLNs and histological analysis. The SLNB procedure is highly invasive and has both a low rate of positive findings (15%) and a high rate of false-negative results (up to 44%). MSOT can reduce unnecessary SLN resection in melanoma patients through non-radioactive SLN detection and non-invasive nodal assessment [1,2].

Detection of SLNs using MSOT

MSOT can resolve both endogenous and exogenous molecules based on their characteristic absorption spectra. Endogenous absorbers such as hemoglobin and melanin can be discerned from exogenous absorbers such as fluorescent contrast agents by spectral unmixing. Injection of fluorescent indocyanine green (ICG) near the primary tumor site results in uptake by nearby lymphatics and draining SLNs can be traced using MSOT without radiation. Stoffels et al. (2015) showed that MSOT was able to detect all SLNs that can be detected by conventional lymphoscintigraphy [1].

INNOVATIVE
Only imaging method to preoperatively assess patients for SLN metastasis.

NON-INVASIVE
Radiation-free SLN detection;
non-invasive assessment of melanoma metastasis in SLNs via transcutaneous imaging.

HIGH PERFORMANCE
Lower detection limit of approx. 4 melanoma cells; achieved a zero-percent false negative rate.

EASY TO USE
Similar workflow as in ultrasound examinations; pre-operative SLN detection and nodal assessment carried out in less than 5 minutes.

IMAGING PROTOCOL

Imaging System	MSOT inVision 128 / MSOT Acuity Echo
Repetition Rate	10 Hz / 25 Hz
Excitation Wavelength	700, 730, 760, 800 and 850 nm.
Processing Methods	Model-based tomographic image reconstruction. Spectral unmixing by a pseudo-inverse algorithm.

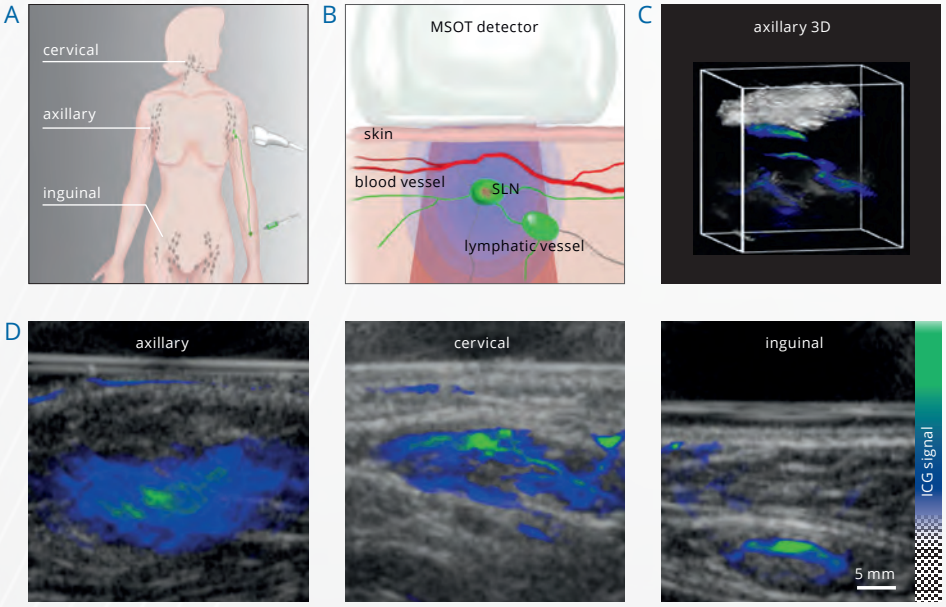

FIGURE 1: SLN detection with MSOT

Panels A and B show a schematic representation of SLN detection by MSOT. Fluorescent contrast agent ICG is injected intradermally at the borders of the primary tumor site and taken up into the lymphatics to highlight the draining SLNs. ICG and melanin are spectrally unmixed from other absorbers such as hemoglobin in neighboring vasculature. Panel C shows the distribution of ICG in lymphatic vessels and the SLN after detection with a 3D detector. Panel D shows representative SLNs detected by ICG-enhanced MSOT in the axillary, cervical, inguinal and regions.

Melanin detection in SLNs

Non-invasive assessment of melanin content in SLNs provides the ability to preoperatively detect metastasis-free SLNs, ultimately reducing the number of unnecessary surgical biopsies. Stoffels et al. [1] demonstrated that MSOT was able to accurately identify metastasis-free SLNs during preoperative scans without a single false negative case, validated by conventional pathology (189 total SLNs). Melanin deposits in metastatic SLNs were also detected by MSOT in preoperative scans, thereby potentially allowing for targeted selection of SLNs for surgical resection and confirmation by conventional pathology.

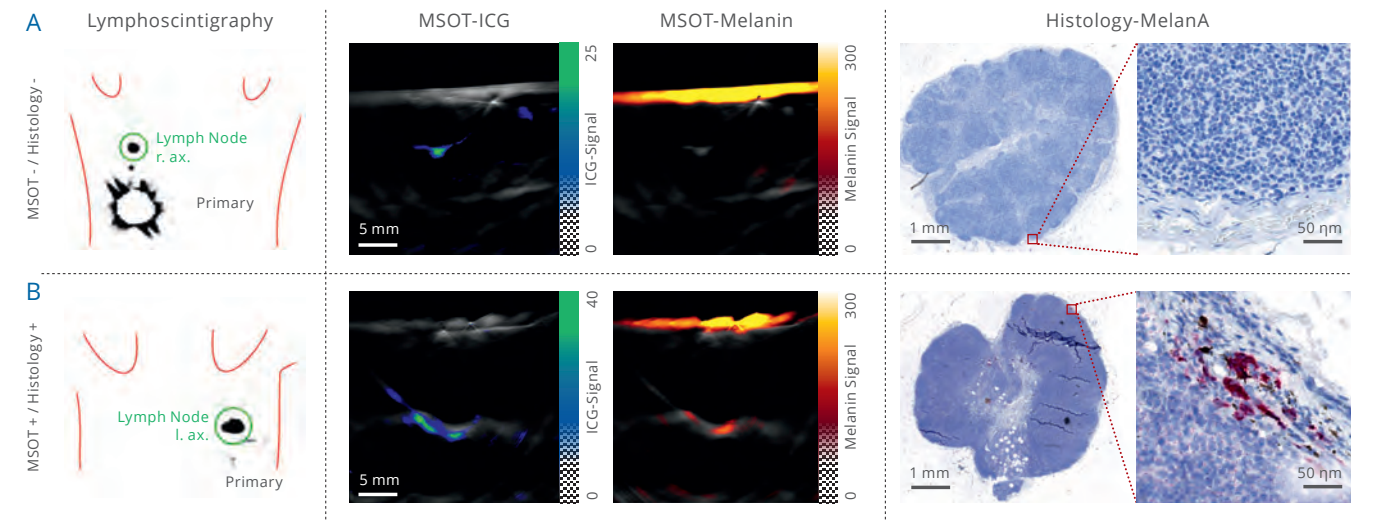

FIGURE 2: Determination of melanin content in SLNs

Shown in Panel A is a representative axial SLN localized by conventional lymphoscintigraphy and by MSOT with ICG contrast (green-blue). This SLN was determined to be negative for the presence of melanin by a preoperative MSOT scan (yellow-red) and this result was confirmed by histology after resection. Panel B shows an axial SLN that tested positive for the presence of melanin deposits in preoperative scans and confirmed by histology following resection.

MSOT limit of detection is just 4 melanoma cells

Agar phantoms embedded with B16 melanoma cells estimate the lower detection limit of MSOT at approximately 4 melanoma cells, thereby potentially enabling the detection of metastasis smaller than 0.1 mm. Additionally, scanning of excised SLNs with MSOT can guide standard histological testing by pinpointing the precise sites of melanin deposits within the SLNs for enhanced detection of nodal metastases.

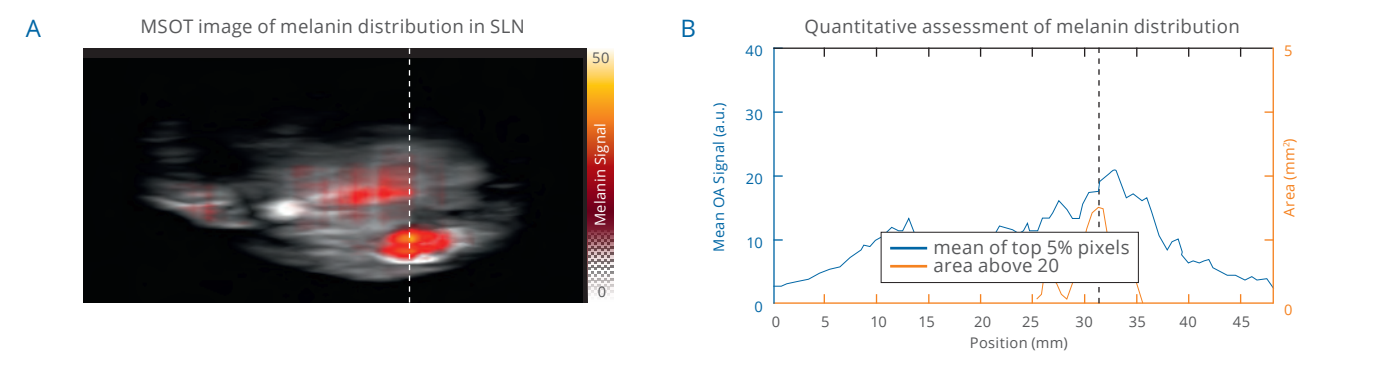

FIGURE 3: *Ex vivo* MSOT-guided histology

Panels A-B highlight the potential for MSOT-guided histological examination by providing melanin distribution information critical for localizing micrometastases in excised SLNs. Panel A shows a 3D MSOT scan of an excised SLN, with corresponding melanin signal plotted against slice position in Panel B.

[1] Stoffels et al., **Metastatic status of sentinel lymph nodes in melanoma determined noninvasively with multispectral optoacoustic imaging**, Sci Transl. 2015 Dec 9;7(317):317ra199.
[2] Stoffels et al., **Assessment of Nonradioactive Multispectral Optoacoustic Tomographic Imaging With Conventional Lymphoscintigraphic Imaging for Sentinel Lymph Node Biopsy in Melanoma**, JAMA Netw Open. 2019 Aug 2;2(8):e199020.