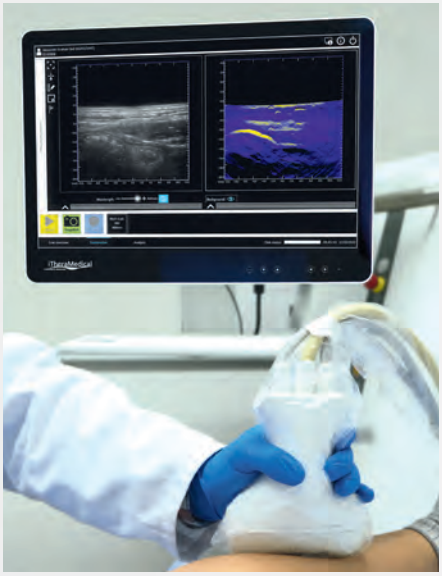

Translational imaging of neuromuscular disease (NMD) using MSOT

NMD is a class of diseases that affect the peripheral nervous system, the neuromuscular junction, or skeletal muscle. They can be progressive, debilitating and/or lethal, posing significant burden on patients, their families and healthcare systems. Currently, the assessment of disease status and therapeutic response relies on functional tests, typically involving a physical challenge (e.g., walking) which is highly variable and unsuitable for young infants and other non-ambulant patient groups. Imaging techniques such as MRI can be used, but can be problematic given the imaging time and the requirement to avoid motion. A clear clinical need exists to objectively and quantitatively monitor the status of NMD patients, particularly to assess treatment response. MSOT imaging has been proposed as an age-independent technology to assess disease status in muscles, non-invasively, with high sensitivity and without the need for patient sedation [1].

Preclinical monitoring of collagen with MSOT

In a translational study by Regensburger AP et al. (Fig. 1), the collagen content of the distal extremities was monitored non-invasively by MSOT imaging without the need for sedation, in both healthy wildtype (WT) and Duchenne muscular dystrophy (DMD) piglets over 4 weeks. The results reveal an excellent sensitivity and specificity of MSOT-derived collagen signal detection to distinguish between healthy and diseased tissue from birth. Validation of the presence of fibrosis biomarkers was performed by immunohistochemistry. An increase of different types of collagens could be found in all DMD piglets from week one of life which strongly correlated with the MSOT findings.

INNOVATIVE
MSOT-based collagen detection could potentially provide a quantitative biomarker for NMD disease progression.

NON-INVASIVE
Handheld transcutaneous imaging, without the need for contrast agents or sedation.

HIGH PERFORMANCE
Real-time visualization of tissue chromophores at high spatial resolution at a depth of up to 3 cm.

EASY TO USE
Similar workflow as in ultrasound examinations; examination carried out in less than 5 minutes.

IMAGING PROTOCOL

Imaging System	MSOT Acuity Echo (Research System)
Repetition Rate	25 Hz
Excitation Wavelengths	680, 700, 730, 760, 800, 850, 920, 1000, 1030, 1064 and 1100 nm.
Processing Methods	Back-projection tomographic image reconstruction; spectral unmixing by linear regression.

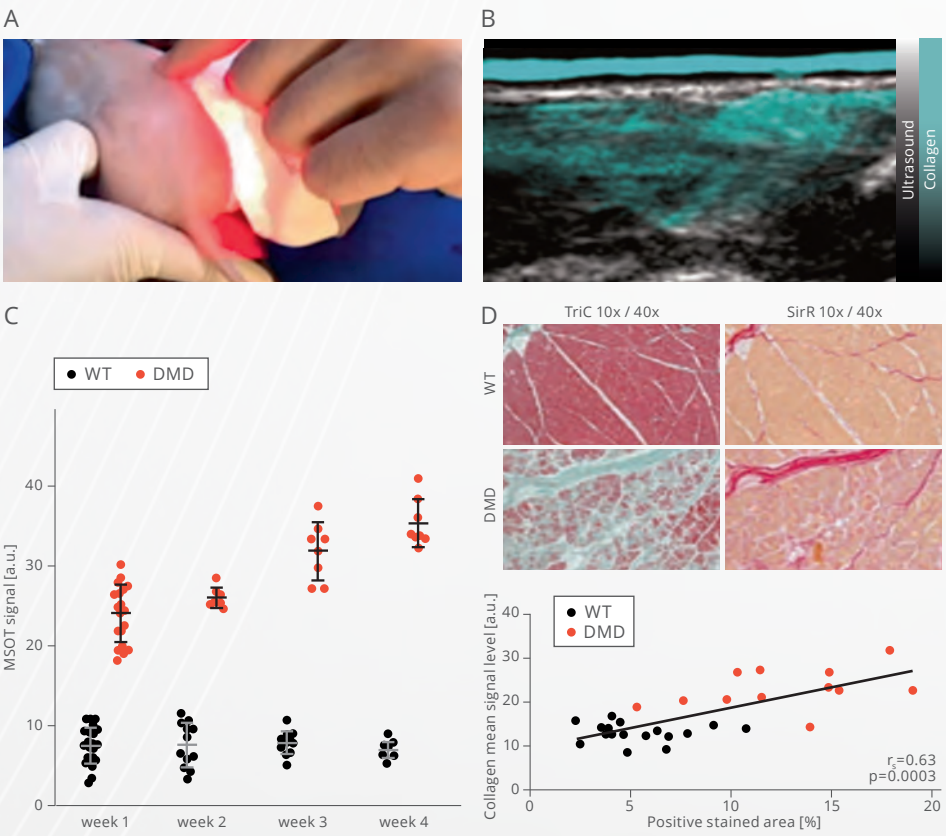

FIGURE 1: Longitudinal assessment of collagen content and *ex vivo* verification in DMD and WT piglets.

Panel A shows MSOT imaging of non-anesthetized piglets. Panel B shows the associated MSOT images with collagen signal overlaid onto ultrasound images. Panel C quantifies MSOT-derived collagen signal in WT and DMD piglets, showing an increase in signal over a period of 4 weeks that was not detectable in the WT group. Panel D shows that collagen content was verified by immunostaining of biopsy samples with Trichrome (TriC) and Sirius Red (SiR), with DMD animals exhibiting higher levels of collagen as compared to WT.

MSOT performance in DMD patients and healthy volunteers

For studying MSOT performance in humans, Regensburger AP et al. (Fig. 2) enrolled 10 ambulatory DMD patients, aged 3 to 10 years and 10 age-matched healthy volunteers (HV) [1]. The results demonstrate the potential for MSOT imaging to distinguish healthy from diseased muscular tissue. Moreover, MSOT signals were significantly correlated to standard physiological and functional tests like the 6-minute walk test (6MWT). These results suggest that MSOT imaging might fill the urgent need for an easy and objective assessment of DMD disease progression and could be evaluated for treatment monitoring in future clinical studies.

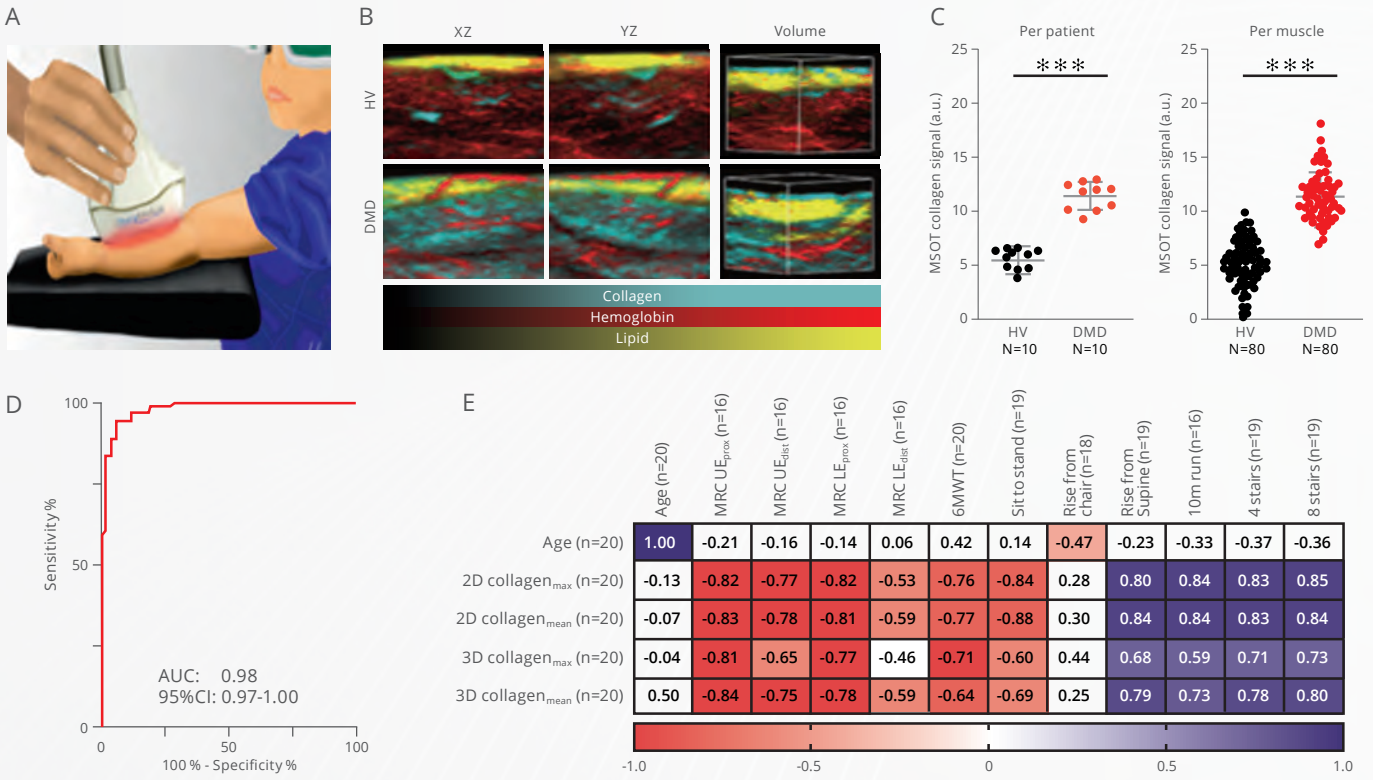

FIGURE 2: Performance of collagen detection via MSOT and comparison to physical examinations in healthy volunteers.

Panel A shows schematically the MSOT detector being used on a patient. Panel B shows a representative MSOT image in a HV vs. DMD patient. Panel C shows quantification of collagen, revealing significant differences in signal strengths between independent muscle regions (n=80 HV, 80 DMD; p<<<0.05) and mean values for each subject (n=10 HV, 10 DMD; p<<<0.05). Panel D shows a ROC curve which illustrates the high sensitivity and specificity of MSOT (independent muscle regions, n=80 HV, 80 DMD; p<<<0.05). Panel E shows correlations to physical examinations, with significant correlations to timed function tests and muscle strength tests (red: negative; blue: positive).

MSOT performance in SMA patients and healthy volunteers

To investigate MSOT performance in a second NMD subtype, Regensburger AP et al. (Fig. 3) enrolled 10 pediatric patients with spinal muscular atrophy (SMA) and 10 gender and age-matched healthy volunteers [2]. The researchers observed a patchy appearance in SMA with increasing loss of MSOT signal depending on the severity of the SMA type, which can potentially be seen as a sign of parallel existence of hypertrophic and atrophic muscle fibers.

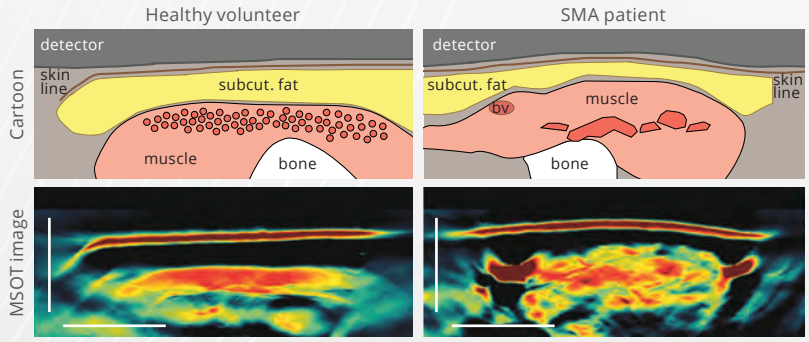

FIGURE 3: MSOT imaging of healthy and SMA-affected muscles.

To illustrate differences between healthy and affected muscles, exemplary images of a HV (left) and SMA patient (right) are shown from the right biceps. The cartoon shows the muscle within its margin. In HV, homogeneous muscle fibers are expected, whereas patchy clusters of hypertrophic and atrophic muscle fibers lead to the moth-eaten pattern in SMA patients. The 800 nm MSOT images reveal patchy signal patterns with alternating high and very low signal intensities in SMA patients in contrast to homogenous signal in healthy muscle.

[1] Regensburger AP et al., **Detection of collagens by multispectral optoacoustic tomography as an imaging biomarker for Duchenne muscular dystrophy**, Nat Med. 2019 Dec;25(12):1905-1915.
[2] Regensburger AP et al., **Multispectral optoacoustic tomography for non-invasive disease phenotyping in pediatric spinal muscular atrophy patients**, Photoacoustics. 2021 Nov 10;25:100315.