CLINICAL INVESTIGATION PLAN

Measurement and imaging capability of a novel medical imaging method, Ultrasound Optical Tomography, based on ultrasound and laser light in human tissue

Short title/acronym: DOIT – Deep Optical Imaging of Tissue

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Revision history

Document version	**Date of Issue**	**Summary of Change**
Version 1	2025-01-29	First issue
Version 2	2025-03-13	Clarification of sponsor Clarification of investigational site including certificate from the site Correction of redundant information section 16.
Version 3	2025-04-06	Addition of one exclusion criteria

Version 4	2025-10-01	Addition of another US frequency. Clarification of examination position and comment about a light compression of the tissue.
Version 5	2026-02-16	Formatting and typos throughout the document. The Swedish synopsis' exclusion criteria regarding keloid formation have been clarified. Increased the number of healthy volunteers to 60, where the additional 20 volunteers are women, intended for breast measurements
Version 6	2026-05-12	Clarification of the increased laser power at 689 nm and references to an updated risk analysis in conjunction with the increased power. Section 2.2 is updated with a rationale for the use of a higher laser power. Section 3 updated to include a summary of risk mitigations.

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Signatures

Sponsor

I am responsible for ensuring that this CIP includes all essential information to be able to conduct this clinical investigation. I will submit the CIP and all other important clinical investigation-related information to the responsible investigator(s) so that they can conduct the clinical investigation correctly. I am aware that it is my responsibility to hold the staff members who work with this clinical investigation informed and trained.

Sponsor's representative's signature Date

Printed name

Principal Investigator

I have read this CIP and agree that it includes all essential information to be able to conduct the clinical investigation. By signing my name below, I agree to conduct the clinical investigation in compliance with this Clinical investigation plan, the Declaration of Helsinki, SS-EN ISO14155:2020 (Good Clinical Practice), and the current national and international regulations governing the conduct of this clinical investigation.

I will submit this CIP and all other important clinical investigation-related information to the staff members who participate in this clinical investigation, so that they can conduct the clinical investigation correctly. I am aware of my responsibility to continuously keep the staff members who work with this clinical investigation informed and trained.

I am aware that quality control of this clinical investigation will be performed in the form of monitoring, audit, and possibly inspection.

Principal Investigator's signature Date

Printed name

Contact information

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Funding and research agreement

The study is an academic investigation where the sponsor and principal investigator is the same person (Sophia Zackrisson). The study is financed by grants from The Swedish Research Council (S Zackrisson PI, Dnr 2022-01140) and the Mats Paulsson Foundation (S Zackrisson PI).

List of used acronyms and abbreviations

Abbreviation	Term/Explanation
ADE	Adverse Device Effect
AE	Adverse Event
CIP	Clinical Investigation Plan
CNR	Contrast to Noise Ratio
CT	Computed Tomography
dB	Decibel
DD	Device Deficiency
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
IB	Investigator Brochure
IEC	International Electrotechnical Commission
IFU	Instructions for Use
MRI	Magnetic Resonance Imaging
NACT	Neoadjuvant Chemotheraphy=pre-operative treatment with chemotheraphy
NIR	Near Infrared Radiation
OI	Optical Imaging

pCR	Pathological Complete Response
PET	Positron Emission Tomography
SS-EN ISO	Swedish Standard - European standard International Organization for Standardization
PP	Per Protocol analysis = including only data from subjects who have completed the clinical investigation completely in accordance with the CIP, with no deviations from the CIP
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
US	Ultrasound
UOT	Ultrasound Optical Tomography
USADE	Unanticipated Serious Adverse Device Effect

1. Synopsis (In Swedish and English)

SVENSK SYNOPSIS	
Bakgrund	Inom medicinsk avbildning används i stor utsträckning röntgenbaserade metoder som innebär att patienter utsätts för strålning. I tillägg, behövs ofta vävnadsprover tas med nålar för att till exempel säkert kunna fastställa eller utesluta en cancerdiagnos. Med vårt projekt utvecklar vi och testar en ljusbaserad (optisk) metod kombinerad med ultraljud som är helt ofarlig för människan, ultraljud-optisk-tomografi (UOT). UOT förutses kunna kunna tränga ned längre in i kroppen för att mäta och avbilda vävnader på ett annat sätt än vad som tidigare var möjligt med liknande metoder. Syftet med detta projekt är att utforska möjligheterna och säkerheten med UOT angående avbildningsdjup och information om vävnadsegenskaper hos friska, frivilliga forskningspersoner, som ett första steg för att förstå teknikens förmåga och begränsningar. Det långsiktiga målet är att i kommande steg utveckla tekniken för klinisk bedömning av cancermisstänkta förändringar med särskilt fokus på bröstcancer och även för bedömning av cirkulationsrubbningar i vävnad.

Prövningsprodukt	Mk1
Kliniskt utvecklinsstadium	Forskning, Pre-Market, Pilot, proof-of-concept
Antal forskningspersoner	60
Studiedesign	Akademisk, öppen, single-center, explorativ proof-of conceptstudie
Inklusionskriterier	• Ålder ≥18 år. • Friska individer utan systemiska eller hudrelaterade tillstånd. • Förmåga att ge informerat samtycke.
Exklusionskriterier	• Graviditet: Även om risken att laserbelysning på huden kan påverka foster är minimal, kommer vi för säkerhets skull att exkludera gravida forskningspersoner. • Hudsjukdomar: Vissa hudtillstånd kan öka risken för skador eller komplikationer vid exponering för laserljus, som exempelvis aktiv rosacea, eksem, hudinfektioner eller tatueringar. • Medicinering: Vissa mediciner kan öka hudens känsligheten för laserljus eller öka risken för biverkningar. Det är viktigt att ta hänsyn till patientens medicinska historia och eventuella pågående behandlingar. • Solbränna: Personer med nyligen exponerad hud för solen kan ha ökad känslighet mot laserljus och kan vara mer benägna att uppleva obehag eller irritation/skada. Detta kan både påverka studiens tillförlitlighet och öka risken för skador på forskningspersonerna. • Historik av keloidbildning eller ärrbildning: För personer med en historia av överdriven ärrbildning kan laserljuset i större utsträckning leda hudirritation. • För mätningar på bröst: tidigare bröstoperation eller bröstcancer: I denna delstudie vill vi endast undersöka helt bröstfriska personer för att kartlägga normalvävnaden.
Vetenskapliga frågeställningar	Primär frågeställning: • Vilket är det största avbildningsdjupet i vävnad med en tillförlitlig signal för UOT? Sekundära frågeställningar:

	• Att undersöka effekten av variationer i UOT-signalen mellan olika individer och positioner samt inom samma individ. Vi kommer att bedöma hur stora dessa variationer är i jämförelse med kontrast-till-brus-förändringarna mellan olika typer av vävnad. • Att övervaka vävnadssyresättning. • Undersökning av UOT-signalens karaktär i normal bröstvävnad. Säkerhetsfrågeställning: Då det är första gången som just denna metod (UOT, med forskningsmaskinen Mk1) används på människa, är den fjärde sekundära frågeställningen relaterad till säkerhet och vi kommer att: • övervaka och rapportera eventuella biverkningar relaterade till mätningarna med UOT
Utfallsmått	Primärt utfallsmått: • Bilddjup: Maximalt vävnadsdjup där en tillförlitlig signal uppnås för båda våglängderna (689 nm och 794 nm). Sekundära utfallsmått: • Signalvariabilitet: Variabilitet i UOT-signaler mellan individer (mätt som standardavvikelse eller variationskoefficient). • Interindividuell variation (t.ex. över olika anatomiska platser eller upprepade mätningar). • Normal vävnadskartläggning: Fördelning av UOT-signaler i arm- och benregioner (medelvärde och standardavvikelse för HbO2 och Hb). • Bröstvävnadskartläggning: Fördelning av UOT-signaler i bröstregioner (medelvärde och standardavvikelse för HbO2 och Hb). • Andel av deltagare där alla avsedda regioner avbildades framgångsrikt). • Funktionell avbildning: Kvantifiering av syresättningsnivåer (HbO2, Hb) på olika djup. Jämförelse av optiska absorptionsvärden för olika vävnadstyper. Säkerhetsutfallsmått:

	• Frekvens och svårighetsgrad av biverkningar (t.ex. hudirritation, obehag).
Förväntat start- och slutdatum	Q2 2025-Q2 2027
Statistiska överväganden	Explorativ studie på friska frivilliga varför analyserna i detta skede inte syftar till att vara tillämpliga på intention-to-treat- eller per protokoll-analyser.

ENGLISH SYNOPSIS	
Background and rationale	In medical imaging, X-ray-based methods are widely used, which means that patients are exposed to ionizing radiation. In addition, invasive tissue samples often need to be taken with biopsy needles to, for example, safely determine or rule out a cancer diagnosis. With our project, we evaluate a light-based (optical) method combined with ultrasound that is completely harmless to humans, ultrasound-optical-tomography (UOT). UOT is predicted to be able to penetrate deeper into the body to measure functions and image tissues than was previously possible with other optical imaging methods. The aim of this project is to explore the capabilities and safety of UOT regarding imaging depth and tissue property information in healthy research volunteers, as a first step to understand the technology's capabilities and limitations. The long-term goal, in future steps, is to develop the technology for clinical assessment of cancer-suspected lesions with a particular focus on breast cancer andfor assessment of circulatory disorders in tissue.
Investigational device	Mk1
Clinical development stage	Research device, Pre-Market, Pilot, proof-of-concept
Number of subjects	60
Design of the clinical investivation	Academic, open, single-center, exploratory proof-of-concept study.
Inclusion criteria	• Age ≥18 years. • Healthy individuals without systemic or skin-related conditions.

	• Ability to provide informed consent.
Exclusion criteria	• Pregnancy: Although the risk that laser illumination on the skin may affect the fetus is minimal, we will exclude pregnant research subjects for safety. • Skin diseases: Certain skin conditions can increase the risk of damage or complications when exposed to laser light, such as active rosacea, eczema, skin infections, or tattoos. • Medication: Some medications can increase the sensitivity of the skin to laser light or increase the risk of side effects. It is important to consider the person's medical history and any ongoing treatments. • Sunburn: Recent sun exposure of the skin may lead to increased sensitivity to laser light and can be more likely to experience discomfort or skin irritation/damage. This can both affect the reliability of the study and increase the risk of injury to the research subjects. • History of keloid formation or scarring: People who have a history of excessive scarring may be more likely to react with greater skin irritation from the laser light. • For breast measurements only: History of breast cancer or surgery. In this project, we aim to map the UOT signal only in healthy breast tissue.
Study objectives	Primary objective: • Determine the deepest imaging depth with a reliable UOT signal in human tissue. Secondary objective(s): • to investigate the effect of variations in the UOT signal between different individuals and positions with the same individual. We will assess how large these variations are in comparison with the contrast-to-noise changes between different types of tissue. • to monitor tissue oxygenation. • investigation of the UOT signal in normal breast tissue Safety objective: • Assess safety, including potential adverse device effects that is deemed as related to the procedure. Particular attention will be given to adverse effects

	on the skin or tissue in immediate proximity of the performed measurements.
Study endpoints	Primary endpoint: • Depth of imaging: Maximum tissue depth where a reliable signal is achieved for both wavelengths (689 nm and 794 nm). Secondary endpoint(s): • Signal variability: Variability in UOT signals between individuals (measured as standard deviation or coefficient of variation). • Intra-individual variability (e.g., across different anatomical locations or repeated measurements). • Normal tissue mapping: Distribution of UOT signals in arm and leg regions (mean and standard deviation for HbO2 and Hb). • Breast tissue mapping: Distribution of UOT signals in breast regions (mean and standard deviation for HbO2 and Hb). • Imaging success rate (percentage of participants where all intended regions were successfully imaged). • Functional imaging: Quantification of oxygenation levels (HbO2, Hb) at various depths. Comparison of optical absorption values for different tissue types. Safety endpoint: • Frequency and severity of adverse events (e.g., skin irritation, discomfort).
Planned duration of the clinical investigation	Q2 2025-Q2 2027
Statistical considerations	Exploratory study on healthy volunteers why the analyses at this stage do not aim to be applicable to intention-to-treat or per protocol analyses.

1. Identification and description of the investigational device

1.1. Description of the investigational device

The device is named Mk1.

In some documentation, variations may occur. Where any of the following names is used, the intended meaning is “Mk1”:

MK1

Mk1 system

Mk1 device

Mk1 UOT device

Mk1 UOT system

UOT system

UOT device

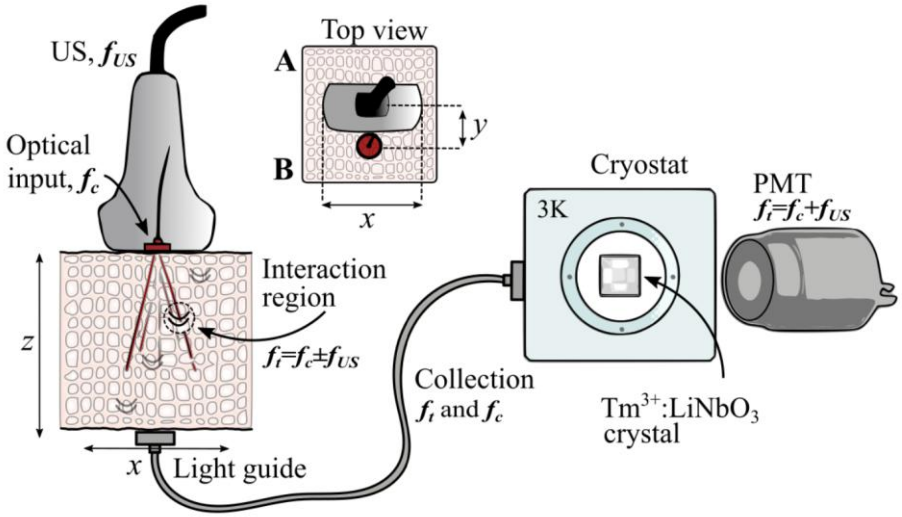


Figure
light are delivered into the target tissue using a US transducer and optical fiber. Photons interacting with the US pulse are frequency-shifted and later separated from the carrier light using a narrowband optical filter. The filter is formed in the cooled rare-earth-ion- doped crystal (Tm3+:LiNbO3) using optical pumping and spectral hole burning techniques. Transmitted photons are recorded using a photomultiplier tube. The y-axis in the top view of the setup notes the direction in which the input light was moved with respect to the transducer to evaluate contrast-to-noise ratio (CNR) dependence on the light-to-transducer distance. Letters A and B (top view) mark the sides of the ultrasound transducer for light illumination experiments published earlier. Image from Bukartė *et al*.(4)

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The schematic representation of this investigational device (Mk1) in **Figure 1** illustrates the setup in transmission mode for ultrasound optical tomography (UOT). Pulsed ultrasound (US) waves and optical light (carrier photons with the frequency f_c) are sent together into the tissue of interest. Carrier photons passing and interacting with the US pulse get frequency shifted (*tagged*) by the frequency of ultrasound (f_{us}) due to the acousto-optic effect ($f_t = f_c \pm f_{us}$). As the tagging efficiency is relatively low (~1 %) and the frequency shift is in the order of f_{us} MHz, which compared to the carrier frequency in THz, is incredibly small (10-5 nm), it is quite difficult

to filter out the photons of interest. We separate the tagged light from the carrier light using a narrowband optical filter formed in a rare-earth-ion-doped crystal using optical pumping and spectral hole burning techniques (2, 3).

A Verasonics ultrasound system (Verasonics Vantage 32LE) with the linear array transducer (Philips, L7-4) generates US pulses with a 4 and 6 MHz tagging frequency. To record an image, 21 parallel consecutive US pulses are emitted along the whole length of the transducer, each displaced by 2 mm along the *x*-axis (horizontal UOT image axis). During the propagation of each US pulse, optical laser pulses at 689 or 794 nm wavelength will be sent into the tissue. The chosen wavelengths are within the first near-infrared tissue optical window (650 to 950 nm) and its suitability for UOT experiments has been demonstrated before (5, 6). Pulses at 689 nm are generated with a thulium sum-frequency fiber-laser system (Precilasers) and pulses at 794 nm are generated from a narrow linewidth tunable continuous wave Ti:Sapphire laser (M squared). Both wavelengths are shaped with a double-pass acousto-optic modulator. To collect only tagged photons, shifted by 4 and 6 MHz from the initial carrier frequency, the narrowband optical filter is created inside a stoichiometric Tm3+:LiNbO3 crystal at the frequency of the tagged first sideband photons (f_t). Transmitted photons are collected with a photomultiplier tube (PMT) and recorded with an oscilloscope. Mk1 is controlled by softwares running on three personal computers where also the measurement information is collected and saved.

For a detailed description of the components of Mk1, please refer to the Investigator Brochure.

1.2. Intended purpose

The Mk1 is an investigational device intended for UOT, a novel imaging method which can image the state of oxygenation of tissues inside the body. It is intended solely for research purposes and not for clinical diagnostic use.

The intended purpose of the Mk1 device is to serve as an investigational system to provide initial UOT measurements and images in-vivo. The performance of the system will be characterized with the aim to provide input data regarding the parameters:

- Imaging depth
- Contrast-to-noise ratio under relevant imaging conditions and depths
- Correlating tissue structures seen in conventional B-scan ultrasound with structures seen in the UOT images.

The purpose of the present clinical trial is, as a first pilot trial in-vivo, to explore the potential of the UOT technique for medical imaging. Thus, the trial will in some sense be exploratory since no images of this type have been acquired previously. Systematic and detailed assessment of clinical performance for medical conditions will be reserved for later clinical evaluation. Consequently, the clinical benefits of using UOT are not known prior to the clinical investigation, but it is plausible that a medical imaging technique that can image the oxygenation of tissues would serve useful diagnostic purposes related to many medical conditions.

Possible areas of medicine where UOT could have benefits in the future are:

- Cancer diagnostics, for tumours with oxygenation characteristics that differ from benign lesions
- Stroke diagnostics and management, for example real-time monitoring of brain oxygenation during thrombectomy procedures
- Diagnostics of heart disease
- Monitoring of brain oxygenation in neonatal care

1.3. Manufacturer of the investigational device

Name: Stefan Kröll, Professor of Physics
Address: Department of Physics, Lund University, Box 118, 221 00 Lund
Contact, phone number: 046-222 96 26

1.4. Model/type

Mk1

1.5. Target population

The Mk1 is an investigational device intended for UOT, a novel imaging method which has the potential to image the state of oxygenation of tissue inside the body. It is intended solely for investigational purposes and not for clinical diagnostic use. The components in contact with the skin are an ultrasound probe and an optical probe. For a detailed description, please refer to the Investigator Brochure.

1.6. Medical or surgical procedures

Not applicable.

1.7. Summary of required training/experience needed

To perform the examination, one experienced operator needs to be present to handle laser and ultrasound. At least one person from the research group that built the Mk1 system will always be present when the system is used for *in vivo* measurements.

For newly employed staff in the research group, training will be provided in person starting from lab entry criteria in addition to Instruction for Use-Mk1. Training will be performed initially on a tissue phantom before any *in vivo* examination. The training should be repeated until the operator is deemed ready to perform an analysis on a human.

The project follows general safety regulations for the Department of Atomic Physics as stipulated by Lund University, which includes a risk analysis for each lab room and existing equipment. All persons who have access to the lab room who handle the Mk1 equipment have approved, documented training and a documented review of safety and privacy routines together with an experienced person.

2. Background and justification for the design of the clinical investigation

2.1. Background

State of the art

Conventional imaging depicts structures and anatomy but with limited functional information. In clinical imaging, conventional techniques rely on depicting structures and anatomy by using X-rays, ultrasound, or magnetic resonance imaging (MRI) (7). By adding intravenously administered contrast compounds (e.g. iodine contrast in X-rays and computed tomography (CT) and gadolinium-based compounds in MRI) limited functional information regarding e.g. tissues and specific lesions (e.g. tumors) can be obtained by mapping how the contrast washes in and out over time in suspicious lesions. Furthermore, so-called functional imaging (revealing physiological activities in living subjects) has come into play where positron emission tomography (PET) is the currently most widely used in clinical medicine (8). The tremendous developments in imaging have clearly moved diagnostics a bit closer to mapping molecular phenomena in tissue but, when a malignancy is suspected, invasive procedures, usually a core-needle biopsy, are still fundamental for a secured, final tissue diagnosis in the pathology lab. This is also true for breast cancer diagnostics where invasive biopsies constitute the final assessment step in cases with suspicion of cancer after imaging with conventional techniques such as digital mammography, 3D mammography (digital breast tomosynthesis and ultrasound. Dynamic contrast enhanced MRI is used for specific indications in breast while PET is not yet used for primary breast cancer diagnosis or staging (9). We are just at the beginning of the era of molecular imaging, and the ultimate goal is to be able to non-invasively monitor all the biochemical processes occurring inside a living human in real time. ***There is hence a great need for further developing molecular imaging methods for clinical translation, filling the gap between structural imaging from conventional methods and functional imaging at cell and tissue level.*** When there is suspicion of breast cancer, several steps of diagnostics need to be passed, often including biopsies. It is equally important to confirm a cancer as to dismiss one in a secure way, especially since many countries offer mammography screening and the number of false positive recalls (no cancer diagnosis) needing biopsies is still a major challenge (10). In the situation where a breast cancer is confirmed, many women receive pre-operative chemotherapy (neoadjuvant chemotherapy, NACT) (11). There is still no perfect imaging modality that accurately can measure the response to treatment, i.e. achieving so-called complete pathological response (pCR)(12, 13). pCR is an established independent predictive factor for breast cancer survival and improved long-term prognosis (14). A huge leap forward in the development of medical imaging is therefore to enable even more tissue specific imaging non-invasively (not needing needle biopsies), without the use of ionizing radiation, and potentially also without the need for intravenous contrast agents. Optical imaging (OI) may provide such information, but the spatial resolution of tissue diagnostics using OI has so far been limited by the strong light scattering properties of tissue. Therefore, OI has since long been held back from translation into the clinic

because of limited ability to image deeper structures. UOT, that we propose to test may overcome this hurdle.

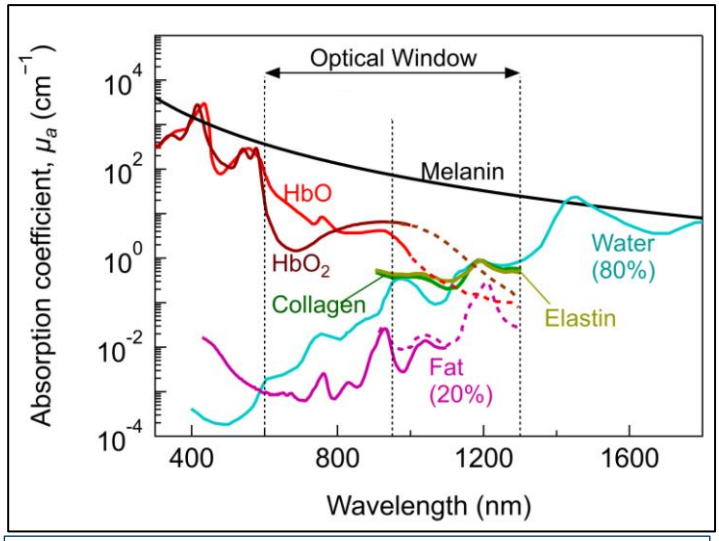


Figure 2. Absorption spectra of the main light absorbing tissue components and the so-called “optical window”. Adapted from Algorri et al. (1).

Optical imaging and endogenous chromophores in tissues

Optical imaging (OI) encompasses a range of techniques using near-infrared (NIR) light (700–1000 nm) and visible light (400–700 nm) to generate molecular, morphologic, and functional information, examining absorption, scattering, and fluorescence properties of cells or tissues (15). Different tissue components have unique wavelength-dependent optical absorption and scattering properties. The diagnostic information obtained from various OI techniques rely on existing differences in the optical properties of healthy tissues, benign and malignant lesions, based on variations in composition of chromophores, molecules able to interact with light (16). Endogenous chromophores naturally present in biologic tissues are, for example, melanin, water, fat, HbO_2 (oxyhemoglobin), and Hb (deoxyhemoglobin). A main target for many OI techniques is distinguishing the two states of hemoglobin (for measurements of oxygen saturation in blood or tissue). In normal conditions, the ratio between oxy- and deoxyhemoglobin is highly stable in tissue, with emphasis on deoxyhemoglobin (normal muscle tissue oxygenation is around 75%) (17). Changes in this ratio may indicate an underlying abnormality, such as hypoxia in tumours caused by e.g. intensively proliferating tumour cells, increased distance to existing vasculature, and inefficient neo-vasculature (18). Melanin, myoglobin, and water are other endogenous absorbers in the range of 600–1000 nm while collagen typically is in the range of 900 to 1700 nm (19, 20). These chromophores are highly relevant for breast tissue diagnostics but can also not be measured and quantified with conventional techniques (only partly depicted). OI hence gives richer molecular information why clinical testing of OI within this area clearly fills an information gap. **Figure 2** shows the

absorption spectra of the main light absorbing tissue components: melanin, oxy-and deoxyhemoglobin, lipid, and water (1). The tissue “optical window” refers to a portion of the visible and near-infrared region (600 – 1400 nm), where the tissue is relatively transparent, so light can reasonably well be transmitted without major absorption to gain spectroscopic information on the tissue interior.

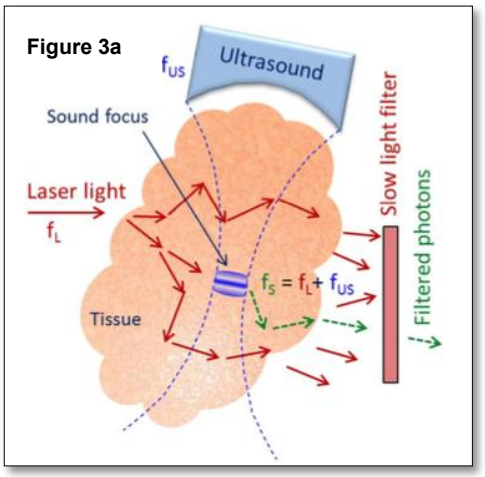
Ultrasound optical tomography – light in and light out, encoded with spatial tissue information by sound.

In this technique, first described in 2003 and during the last decade uniquely developed by groups at Lund University (Kröll, S. *et al.*), light and ultrasound are combined for optical imaging deep inside tissue (21-23). Optical tissue imaging can provide biomolecular contrast that is not readily reachable with other imaging modalities. We focus on developing a technique for instantaneous measurements of blood oxygenation which have significant influence in many areas like tumour detection, myocardial infarction, or stroke. However, the technique can in addition to tissue oxygenation also in general measure the optical absorption and scattering properties of tissue, and this is what we will develop and further capitalize on in this project.

In general, the spatial resolution of tissue diagnostics using optical techniques is limited to a few cm due to the strong scattering properties of tissue. Using quantum designed filter structures with large acceptance angle that are many orders of magnitude narrower than any other large acceptance angle filters, this technique can image deeper into tissue with significantly better contrast-to-noise than other optical techniques (24-26).

A description of the UOT principle is illustrated in **Figure 3a and 3b**. By analyzing the frequency shifted light only, a spatial resolution equal to the ultrasound focus can be obtained. This technique is called ***Ultrasound Optical Tomography*** (**UOT**). A critical factor in UOT is the ability to discriminate between the light frequency-shifted by the ultrasound and the much stronger non-frequency-shifted light.

The absorption of ultrasound in tissue increases with increasing ultrasound frequency, and therefore the ultrasound frequency f_{US} preferably should be just a few MHz. The small frequency shift is why previous attempts to develop UOT techniques have had limited success: conventional filtering techniques are either not narrow enough to suppress only the carrier or has a very small acceptance angle – a serious drawback as light exiting tissue propagates in all directions.



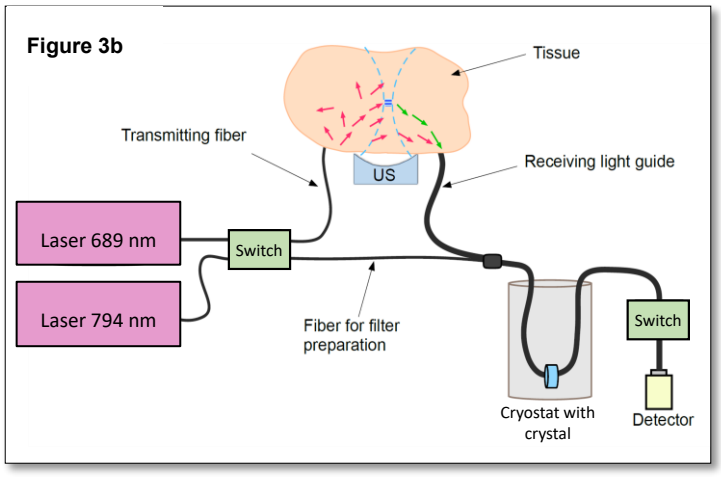


Figure 3a. Principles of ultrasound optical tomography, **UOT**. When an ultrasound pulse with frequency f_{US} is focused in tissue, light with frequency f_L (red arrows) traversing this small volume can be tagged by having its frequency shifted to $f_S = f_L + f_{US}$ (dashed green arrows). By analyzing just the frequency shifted light, a spatial resolution equal to the ultrasound focus can be obtained. This enables us to carry out optical tissue diagnostics at significantly larger depths than with other techniques, estimated up to 5-10 cm. **Figure 3b** shows a schematic setup of an UOT system. (US= ultrasound)

The Quantum Information Group at the Division of Atomic Physics, Department of Physics at Lund University, has world leading experience in creating narrowband (1 MHz) filters with high (~50 dB) suppression as well as a large acceptance angle. These so–called *Slow Light Filters* are created in inorganic crystals doped by rare-earth ions, where laser light is used to semi-permanently transfer ions to non-absorbing states. The filters are created by "burning" a spectral hole in the absorption profile of the crystal at the frequency f_S so that it transmits this frequency but absorbs the unwanted background light at the frequency f_L. An added and important effect of these filters is that light propagating at the transmitted frequency, f_S, will be slowed down by several orders of magnitude. In this way, any remaining background from the carrier frequency after the filter can be suppressed using time gating.

In terms of combined suppression of non-frequency shifted light and acceptance angle for the frequency shifted light, slow-light filters outperform currently existing filters by several orders of magnitude, in turn enabling orders of magnitude better contrast for tissue imaging.

- ***In summary, UOT*** is an emerging technology which is predicted to reach much deeper than previous optical imaging techniques and to have an order of magnitude better signal-to noise or contras-to-noise already at 1 cm depth and 2 orders of magnitude stronger signal at 2 cm depth (27). It is unique in the sense of being an optical imaging technique expected to be able to utilize light transmitted through 10 cm of tissue, a feature not present in any other optical imaging technique. In this first proof-of-concept project we will primarily assess the imaging depth and imaging quality in muscle and breast tissue to be able to adapt the system to better performance before moving to early clinical trials. Muscle tissue oxygenation is well studied using optical techniques and is therefore suitable for validation of UOT measurements. Breast is expected to be an excellent first clinical performance test model since the organ has a rather superficial

and accessible anatomical position and offer many conventional techniques for cross-referencing. If UOT is successful in breast, it may be further developed for applications in other parts of the body and organs.

2.2. Evaluation of results of prior testing, assessments and clinical investigations

UOT was first described in 2003 (23) and was then tested on humans (arm). However, the way the output signal was collected is different from our technique with the Mk1, where we can reach much deeper to collect tissue information because we can detect much weaker signals. The difference is that we treat the light that comes out of the body differently than Lev and Sfez in 2003. However, what happens in the body with light and ultrasound is the same as for UOT. This is also true for other clinically established optoacoustic techniques and there are commercially available systems for e.g. breast cancer diagnostics approved for clinical use https://senomedical.com/newsroom/press-releases/P18

Similar techniques using laser and ultrasound are hence approved for clinical use and are used in other clinical trials (including optoacoustics as mentioned above). We therefore believe that animal experiments should not be conducted with UOT, as it is a similar technique, but with the advantage of probably being able to reach deeper into the tissue and where the only difference is which output signal is captured outside the body (sound or light).

The strength of the UOT signal depends on the total number of photons and how many of those that have interacted with the ultrasound field. For a better contrast-to-noise ratio, hence a better image quality, as high laser power as possible, but below the maximum permissible exposure (MPE) on the skin, is desirable. A way to stay below the MPE, while increasing the maximum emitted power, is to spread out the light over a larger area. For eye safety, protective eyewear is of high importance.

Below follows a description of our preparatory work.

System setup and testing

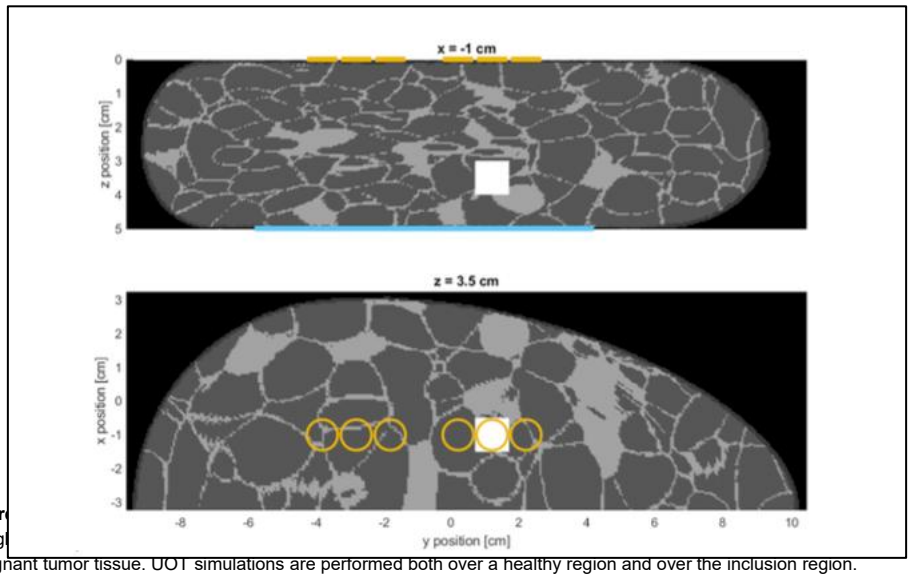
The team at the Department of Physics has developed and tested the transportable system prototype to be used in this project, the Mk1 (3, 28-30). The observed signals have been fully consistent with the calculated signal levels (24-26). No free parameters were used when comparing theoretical calculations and experimental data, which gives us great confidence in our ability to predict signal levels and judge the possible performance of the technique.

Computer modelling of UOT acquisition

A preliminary computer model of UOT data acquisition, using Monte Carlo simulations, has been developed by the research team. Simulated photons are scattered through the breast tissue model to record their paths to the detector. Separately, tissue absorption is calculated along the recorded photon paths (which may be repeated for various tissue distributions). Breast anatomy is simulated using our open software OpenVCT (31, 32) expanded by realistic

Perlin noise-based tissue models (33, 34) and was presented at and selected the best live demo workshop at the SPIE Medical Imaging Conference in San Diego, USA 2023 (35). The simulation allows us to test the effect of tumor size, location, and structure on the UOT signal, with the aim of distinguishing between benign and malignant findings.

In a collaborative follow-up work the physics and radiology team presented at the 2024 Conference on Virtual Imaging Trials in Medicine, Duke University, USA 2024 that UOT is a method capable of distinguishing malignant tumor tissue from benign glandular tissue, despite the natural variations within a breast or between breasts with different volume breast densities (36), **Figure 4 and 5**. Current work focus on the reconstruction algorithm to be improved and optimized to better utilize all information contained in the UOT signal to achieve an even better spatial resolution and a higher sensitivity to distinguish differences in absorption coefficients.



Figur
fibrog
malignant tumor tissue. UOT simulations are performed both over a healthy region and over the inclusion region. For each region, light is emitted from three 1 cm diameter sources (yellow lines) and for each source, light is collected by a light guide centered right underneath the light source and with a 4 cm diameter (the blue line in the top graph covers the entire area used for detection) (36).

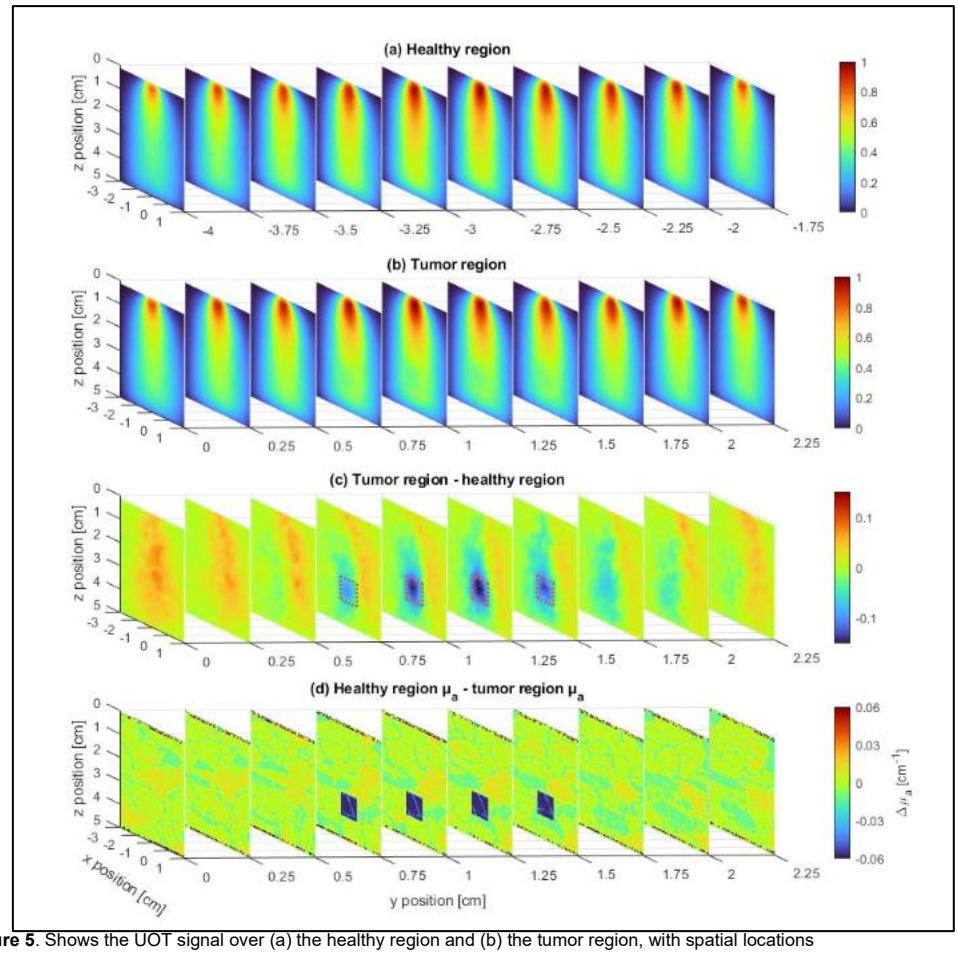


Figure 5. Shows the UOT signal over (a) the healthy region and (b) the tumor region, with spatial locations indicated in Fig. 4. The largest variation in signal is due to the difference in photon flux throughout the tissue. (c) The difference between the two signals indicates differences in the underlying optical properties, where most notably the tumor inclusion (red dashed box) results in a lower UOT signal. (d) Shows the true difference in absorption coefficients between the two regions (36).

With simulations using the diffusion model it is shown that a spread-out area, necessary with increased laser power, increases the image quality closer to the light source as the light field becomes more even. As a result, the contrast-to-noise ratio can stay high, even at the edge of the image area. **Figure 6** compares a 1 cm line light source with a 5 cm line light source.

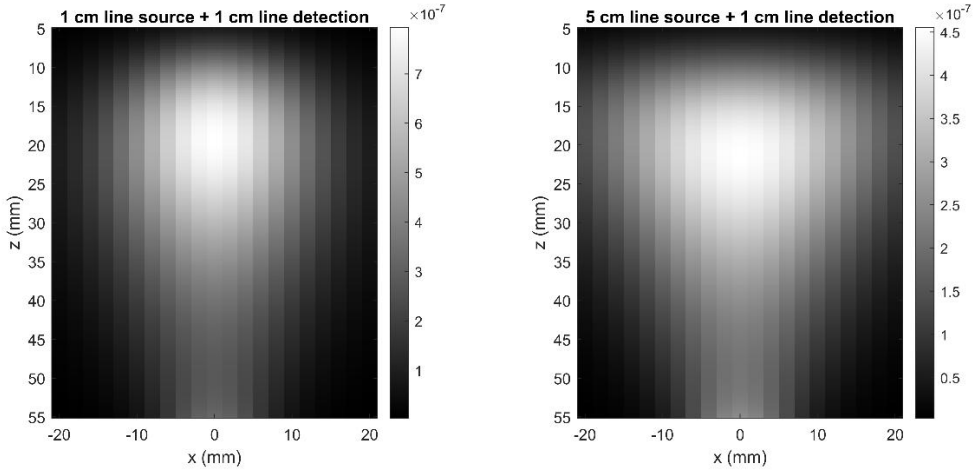
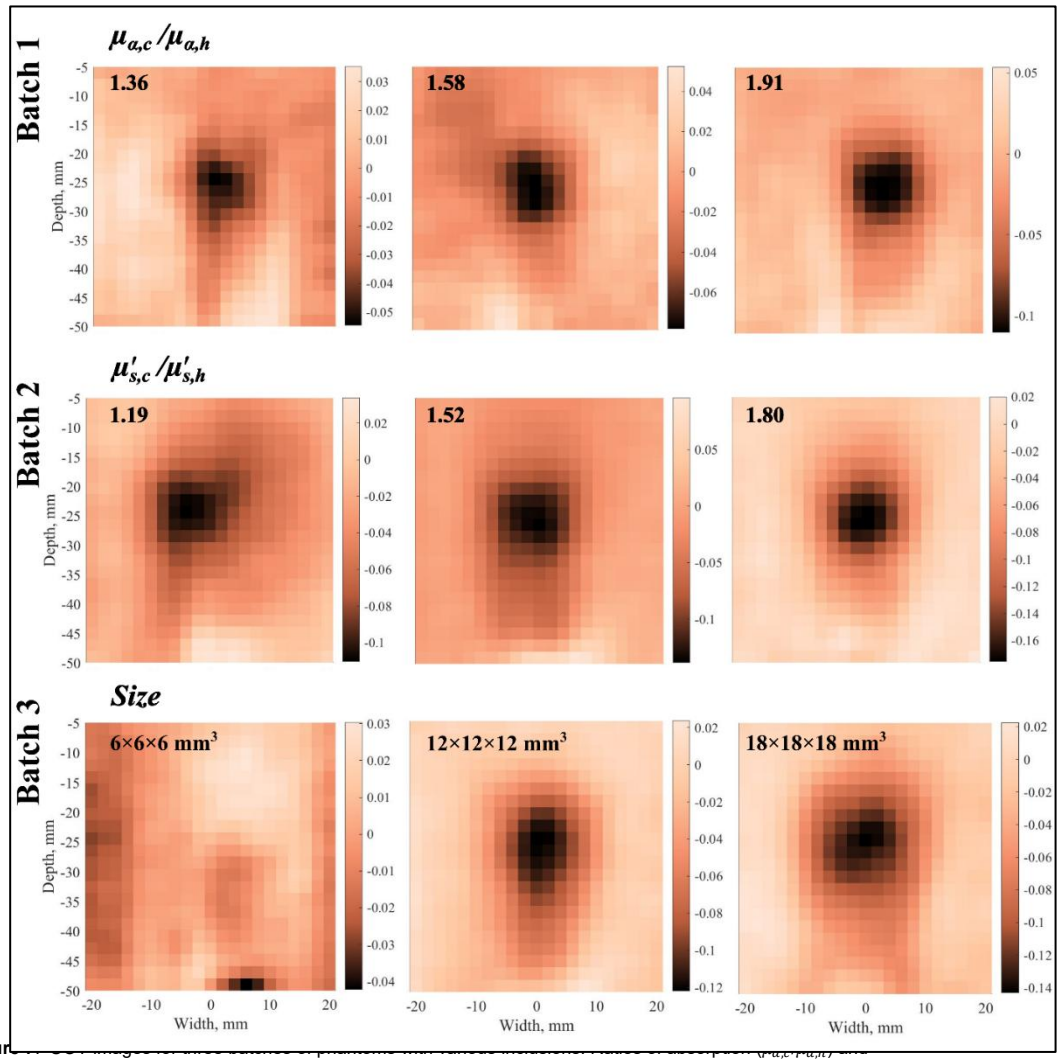


Figure 6 Comparison between a 1 cm line source and a 5 cm line source. Both UOT images are simulated using the diffusion model with estimated scattering and absorption, representative of real human soft tissue. The light source is placed at the top of the image, and light drain at the bottom. In the right image, the light intensity has a more even illumination at the upper part. The color bar is in arbitrary units.

UOT experiments in artificial tissue phantoms

The current version of the UOT system is in use in various experiments to image artificial tissue phantoms mimicking 4-5 cm thick breast tissue containing inclusions with optical properties corresponding to breast cancer tumours using light at 794 nm, yielding very promising results (37). As a first step in characterizing and probing the abilities of the UOT for breast cancer imaging, we have successfully produced tissue-mimicking agar-intralipid phantoms with cancer-mimicking inclusions varying in absorption, scattering, and size. We have shown that using this technique, we are able to image 12×12×12 mm^3 and larger-size cancer-like inclusions with CNR values ranging from 3.87 to 6.24. Moreover, there is a high chance that with several improvements, it is possible to image even smaller inclusions since the CNR value of 6×6×6 mm3 inclusion was above one and reached 2.21, **Figure 7, (38)**.



Figur reduced scattering ($\mu'_{s,c}/\mu'_{s,h}$) coefficients and size of the inclusions in Batch 3 are presented at the top left corner of each UOT image. No normalization was done before plotting each image and the color scales represent values obtained after subtracting two normalized UOT signals (38)

.

The agar-intralipid phantoms described above work well for the optical parts of the UOT but lack acoustic properties. Therefore, we have now established a protocol for copolymer-in-oil tissue-mimicking phantoms with both optical and acoustic properties for optical tuning to be used in upcoming UOT experiments (39). With the copolymer-in-oil mimicking phantom, data was acquired with a spread out, line source (**Figure 8**). It shows that a larger light field leads to a more even distribution of the light field close to the illumination area, in agreement with the simulations in **Figure 6**.

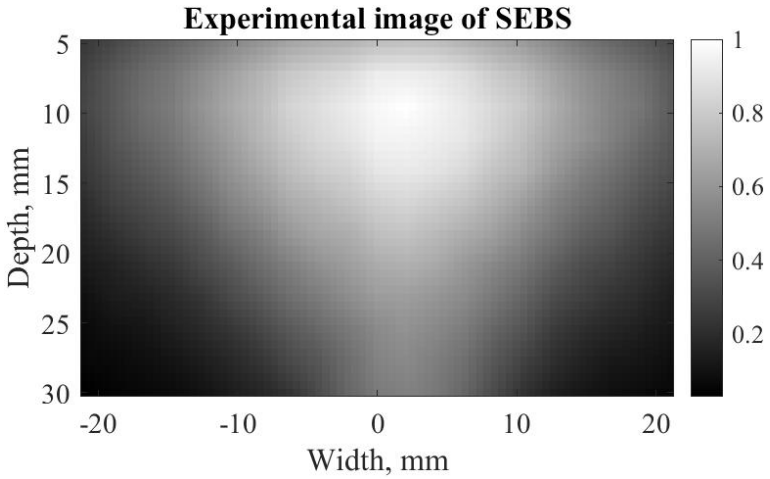


Figure 8 Image acquired with a copolymer-in-oil tissue mimicking phantom (styrene-ethylene/butylene-styrene (SEBS)). The light field is more even close to the top part where the illumination area is, in agreement with the simulations performed for the same light source and drain geometry.

Conclusion

In conclusion, the technique behind UOT and the capability of the current Mk1 system has been described as well as proven in several virtual and physical phantom studies and is ready to be tested in humans. This will inform us whether the targeted imaging depth of 10 cm through tissue is feasible also *in vivo*. In parallel, further development and optimization work with e.g. a second wavelength of 689 nm as well as testing with other filters for faster signal recording will be pursued in phantom experiments.

2.3. Evaluation of clinical data

There is convincing clinical data from trials that optoacoustic techniques can differentiate e.g benign and malignant breast lesions (40-42). The major challenge into actual clinical translation and use is that the previously described techinques only reach around 2 cm deep in the tissue. UOT is expected to be able to utilize light transmitted through 10 cm of tissue.

2.4. Description of the clinical development stage

Mk1 is an investigational device to be used in an exploratory pilot study

.

3. Risks and clinical benefits of the investigational device and clinical investigation

3.1. Expected clinical benefits

There are no direct benefits for the healthy volunteers participating in the project. In the long term and later development stages of the device, the following clinical benefits are relevant:

- Non-invasive imaging with potential to reduce reliance on biopsies.
- Enhanced understanding of tissue oxygenation and composition.
- Potential applications for early cancer detection and treatment monitoring.

The purpose of the present clinical trial is as a first pilot trial in-vivo to explore the potential of the UOT technique for medical imaging. Thus, the study will be exploratory since no images of this type have been acquired previously. Systematic and detailed assessment of clinical performance for medical conditions will be reserved for later clinical evaluation. Consequently, the clinical benefits of using UOT are not known prior to the clinical investigation, but it is plausible that a medical imaging technique that can image the oxygenation of tissues would serve useful diagnostic purposes related to many medical conditions.

Possible areas of medicine where UOT could have benefits in the future are:

- Cancer diagnostics, for tumours with oxygenation characteristics that differ from benign lesions
- Stroke diagnostics and management, for example real-time monitoring of brain oxygenation during thrombectomy procedures
- Diagnostics of heart disease
- Monitoring of brain oxygenation in neonatal care

3.2. Anticipated adverse device effects

- **Laser Exposure:** Potential thermal effects, mitigated by compliance with International Standard on Laser Safety (IEC 60825-1). The laser belongs to class 4, and incorrect usage of protective eyewear can lead to eye-damage. Safety precautions are made – the laser is enclosed to be degraded to class 1. All personnel and volunteers must wear protective eyewear at all times.
- **Ultrasound Exposure:** Minimal risk of tissue heating or mechanical effects. Risk analysis confirms that UOT parameters fall within safe ranges for human use.

For detailed information, please refer to Investigator Brochure. A complete laser safety assessment is found in the Appendix III Mk1 Laser safety assessment document.

3.3. Risks associated with participation in the clinical investigation

Please refer to Investigator Brochure. There is a small risk that we incidentally will see lesions in the breast with the ultrasound probe, that are not known to the participants (healthy volunteers) and that will need medical attention.

3.4. Possible interactions with concomitant medical treatments

Subjects on medical treatment that can increase the sensitivity of the skin to laser light or increase the risk of side effects will not be included.

3.5. Steps to be taken to control or mitigate risks

Please refer to Investigator Brochure.

In case of incidental findings in the breast (see 4.3), we will recommend the participant to consult health care further investigations.

3.6. Rationale for benefit-risk ratio

The participating researchers have many years of experience in research studies where the interaction between light and tissue is investigated to verify theoretical models of light scattering and absorption in tissue including diagnostic methods. The PI/Sponsor, Sophia Zackrisson is a clinically active radiologist in the field and, has in recent years been involved in studies with so-called photoacoustics together with researchers and doctors at the Department of Ophtalmology, Skåne University Hospital, Lund, Sweden. Photoacoustic imaging is a method that is similar to UOT and that also combines pulsed laser light and ultrasound. Together we have developed a clinical routine with safety aspects for photoacoustics, which is directly transferable to UOT (43).

In case of incidental findings, the PI/sponsor Sophia Zackrisson will evaluate the findings and whether the research subject should be recommended to consult healthcare for further investigations.

Clinical collaborator Dr Emilie Krite Svanberg, has a long (approx 15 years) scientific experience within the field of spectroscopic methods for measurements on tissue oxygenation and lung monitoring.

The project follows general safety regulations for the Department of Atomic Physics as stipulated by Lund University, which includes a risk analysis for each lab room and existing equipment. All people who have access to the lab room who handle the equipment have approved, documented training and a documented review of safety routines together with an experienced person. See updated risk assessment for the laser system, Appendix III Mk1 Laser safety assessment.

To secure the research person’s integrity and privacy during the informed consent process and the measurement procedures, there will be a sign on the lab room door with information of occupancy/free. Only the person conducting the measurements and the PI will be present in the room.

During the UOT procedure, the skin will be exposed to laser energy levels that are below those stated in the International Standard on Laser Safety (IEC 60825-1)(44). Use of safety eyewear by all people present in the investigation room will be compulsory, as are warning signs and a non-reflective environment. All personnel present in the investigation room have documented training in laser safety.

The Mk1 UOT system utilizes laser and ultrasound modalities that come into direct contact with the patient. By employing near-infrared wavelength at794 nm and visible 689 nm, which are non-ionizing radiation, the primary risk involves thermal heating due to the tissue absorption of the light. For ultrasound, the primary risk includes potential tissue heating and mechanical effects, such as cavitation or tissue disruption. The 689 nm radiation is planned to operate at a higher power than the 794 nm. To mitigate risks, the illumination area is made larger, please refer to the investigation brochure and the laser safety assessment.

To address these risks comprehensively, a thorough risk analysis has been conducted. This analysis evaluates all identified potential hazards associated with the use of the Mk1 system, and appropriate risk mitigations have been implemented. This risk analysis is documented in the Investigator Brochure, which details the identified risk, assessment methods, and specific risk reductions. These strategies encompass assessing the light and ultrasound output, checking electrical safety, the use of personal protective equipment, and necessary equipment provided to minimize the risk. Additionally, a clear Instructions For Use document is provided. The residual risks have been evaluated and a benefit-risk analysis has been performed. With a higher laser power, with the implemented risk mitigation, the risk level remains unchanged. Higher laser power leads to no increased benefit to the test subject but increases the image quality.

The conclusion of the benefit-risk analysis is that the possible benefits of evaluating UOT on human subjects outweigh any remaining risks. The identified residual risks are listed in **table 1**.

Table 1. *Categorised* r*esidual risks*

Type of hazard	**Effect**	**Analysis**
Electrical, fire, explosion and contamination hazards	Damage to personnel or subject	These hazards are common for all electrical medical devices, and the risks are regarded as acceptable if the device complies to the general IEC safety standards.
Unintentional laser emission	Eye damage	These hazards are common for all Class 4 medical laser equipment,

		and the risks are regarded as acceptable if the device complies with safety standards relevant for laser products, i.e. IEC 60825-1. All personnel and volunteers present in the investigation room must always wear protective eyewear while the laser emission is on.
Too high laser emission	Heating of skin	The laser power is spread out over a large area so that the power density on the skin falls below MPE. Even if the laser control mechanisms fail, the system cannot emit laser power above the safety limits.
Too high ultrasound emission	Tissue damage	Even if the ultrasound control mechanisms fail, the system cannot emit ultrasound power above the safety limits

By implementing the risk mitigation measures, along with thorough training and adherence to established protocols, we strive to create a safe research environment while ensuring scientific accuracy and participant welfare and integrity. Details of the risk management are to be found in the Investigator Brochure.

5. Objectives and hypotheses of the clinical investigation

5.1. The purpose of the clinical investigation

The purpose of the study is as a first pilot trial *in vivo* to explore the potential of the UOT technique for medical imaging in healthy volunteers.

The intended purpose of the Mk1 device is to serve as an investigational system to provide initial UOT measurements and images *in vivo.* The performance of the system will be characterized with the aim to provide input data regarding the parameters:

- Imaging depth
- Contrast-to-noise ratio under relevant imaging conditions and depths
- Correlating tissue structures seen in conventional B-mode ultrasound with structures seen in the UOT images.

- Safety

5.2. Objectives

Primary objective

Determine the deepest imaging depth with a reliable UOT signal in human tissue.

5.2.1. Secondary objective(s)

1. UOT measures the optical absorption and optical scattering properties of tissue. The first secondary outcome is:

5.2.2.

- to investigate the effect of variations in the UOT signal between different individuals and positions within the same individual. We will assess how large these variations are in comparison with the contrast-to-noise changes between different types of tissue.

Mapping of normal tissue anatomy in relation to the UOT signal will give a fundamental understanding of the clinical potential of the new system. The normal background signals, with regards to e.g., sex, skin coloring and the amount of subcutaneous fat need to be well understood before moving to imaging of cancerous tissue in future steps.

2. Cancerous tissue often exhibits hypoxia compared to normal tissue. One major biomarker in optical imaging is the ability to measure blood and tissue oxygenation. The second secondary outcome is therefore

- to monitor tissue oxygenation.

Through UOT measurements we will monitor HbO_2 (oxyhemoglobin) and Hb (deoxyhemoglobin) in the tissue, at different time points.

3. One long-term goal is to develop the technique to be used in clinical assessment of lesions with suspicion of malignancy, with special focus on breast cancer. Therefore, mapping of normal female breast tissue anatomy in relation to the UOT signal is essential. The third secondary outcome is

5.2.1.

- investigation of the UOT signal in normal breast tissue

Safety objectives

Assess safety, including potential adverse device effects that are deemed as related to the procedure. Particular attention will be given to adverse effects on the skin or tissue in immediate proximity to the performed measurements.

Data on all adverse events will be collected. Data to be recorded are type of event, start and stop dates, highest severity grade, seriousness, action taken, outcome, and causality. An overview of all adverse events, including highest severity grade, causality, seriousness, and adverse events leading to deaths or withdrawals, will be presented. Incidence of all adverse events will be presented by system organ class and preferred term.

5.3. Hypotheses

Primary hypothesis

Primary Hypothesis: UOT can provide reliable imaging signals at depths greater than current optical imaging techniques.

5.3.1.

Secondary hypothesis(es)

5.3.2.

Secondary Hypothesis: UOT signals correlate with tissue composition and oxygenation, showing minimal variation among healthy individuals and is safe to use.

6. Design of the clinical investigation

6.1. General information

This is an academic, single-center, exploratory proof-of-concept study. The investigational device, Mk1, will be used to image arms, legs, and breast tissue of healthy participants. No control group or comparator is to be used because of the exploratory design.

6.2. Endpoints

This is an exploratory, proof-of-concept study.

6.2.1. Primary endpoint

- Depth of imaging: Maximum tissue depth where a reliable signal is achieved for both wavelengths (689 nm and 794 nm).

6.2.2. Secondary endpoint (s)

- Signal variability: Variability in UOT signals between individuals (measured as standard deviation or coefficient of variation).
- Intra-individual variability (e.g., across different anatomical locations or repeated measurements).

- Normal tissue mapping: Distribution of UOT signals in arm and leg regions (mean and standard deviation for HbO2 and Hb).
- Breast tissue mapping: Distribution of UOT signals in breast regions (mean and standard deviation for HbO2 and Hb).
- Imaging success rate (percentage of participants where all intended regions were successfully imaged).
- Functional imaging: Quantification of oxygenation levels (HbO2, Hb) at various depths. Comparison of optical absorption values for different tissue types.

6.2.3. Safety endpoint

Exposure to the investigational device Mk1 during measurements

- Frequency and severity of adverse events (e.g., skin irritation, discomfort).

6.2.4. Description of the comparator (applicable if the comparator is a medical device)

Not applicable.

6.3. Subjects

6.3.1. Inclusion criteria

The inclusion criteria for the study include that the research subjects must be over 18 years of age and be in good health and without any ongoing disease or other condition that could affect the results of the study.

- Age ≥18 years.
- Healthy individuals without systemic or skin-related conditions.
- Ability to provide informed consent.

6.3.2. Exclusion criteria

To minimize the risk of harm or unwanted side effects, the study should exclude research subjects due to:

- Pregnancy: Although the risk that laser illumination on the skin may affect the fetus is minimal, we will exclude pregnant research subjects for safety.
- Skin diseases: Certain skin conditions can increase the risk of damage or complications when exposed to laser light, such as active rosacea, eczema, skin infections or tattoos.
- Medication: Some medications can increase the sensitivity of the skin to laser light or increase the risk of side effects. It is important to consider the person's medical history and any ongoing treatments.
- Sunburn: People with recently exposed skin to the sun may have increased sensitivity to laser light and may be more likely to experience discomfort or irritation/damage. This

can both affect the reliability of the study and increase the risk of injury to the research subjects.

- History of keloid formation or scarring: People who have a history of excessive scarring may be more likely to react with greater skin irritation from the laser light.
- For breast measurements only: History of breast cancer or surgery. In this project, we aim to map the UOT signal only in healthy breast tissue.

6.3.3. Investigation population

Total: 60 healthy volunteers

- 30 participants for arm and leg measurements (approx. 50/50 male-to-female ratio).
- 30 females for breast measurements.

For the recruitment of research subjects for this study, we will advertise the study primarily among students and staff at Lund University. Posters and digital notice boards will be distributed across the university to raise awareness, and meetings will be held to inform about the study. The recruitment process will be transparent and follow ethical guidelines to ensure the participation of research subjects on a voluntary basis (Bilaga 2). Since the study is highly exploratory, a range of healthy volunteers of different ages, genders, and body compositions are deemed to be sufficient to be representative in relation to the outcome of measures at this research stage. No vulnerable subjects will be included.

6.3.4. Criteria and procedures for subject withdrawal or discontinuation.

By signing the consent form, the research subjects give their informed consent to participate in the study and for their data to be processed according to applicable laws and research ethics principles. The consent process emphasizes the research subjects' rights and the possibility to withdraw their participation at any time without consequences. Since the follow-up is very short, within 24h, we believe that there will be none or very few subjects that are lost to follow-up.

6.4. Methods to minimize bias

In this exploratory proof of concept study including healthy volunteers of both genders with very general outcomes on tissue properties, we do not expect any major biases. The proposed project parts should hence be considered as proof-of-concept studies. The number of healthy volunteers proposed are planned to ensure representation of variety of different tissue characteristics. We plan to include 30 healthy volunteers, both males and females in about 50/50% proportions for arm and leg measurements and 30 females for breast tissue measurements, so in total 60 persons. Mostly descriptive statistics will be used.

6.5. Unblinding

Not applicable.

6.6. Description of the clinical procedures and diagnostic methods relating to the clinical investigation

Table 2. Flow chart of the investigation.

Procedure	*Screening* *Day1 Inclusion visit*	*Visit 1* *Day 1 Baseline*	*Follow-up* *Day1 + 24h*
Incl/exclusion criteria	√		
Informed consent	√		
Medical history/ concomitant medications	√		
Measurements		√	
Adverse Events		√	√

First, the research subject will fill in a form with demographic and anthropomorphic background information such as age, gender, weight, length etc. After that, we will measure the percentage of fat on the skin using a slide caliper, at the place where the measurements will be performed. This is done as laser light is absorbed differently in different types of tissue, which means that there is a possibility that different fat percentages can affect the result. This is followed by an assessment of the research person's skin tone according to the Fitzpatrick scale (1-6) (45), so that we can get an idea of the effect of skin tone on UOT signal. Here it will also be ensured that the research subject has no skin conditions that could affect the results. The research subject will then sit in a comfortable and rested position with the extremity or breast resting on a supporting plate. All the people in the room, including the research person, will wear protective eyewear. After calibration, UOT measurements will be performed on extremities (both men and females) and on breasts (females). A water-based coupling gel will be used between the ultrasound and the skin as in clinical routine ultrasound. The laser light guide and ultrasound probe will be placed on the skin of the extremities or breast at different predefined positions according to a measurement scheme. A light compression will be applied to ensure good contact between transducer and the skin and to avoid motion artifacts. Pulsed laser light and US waves are transmitted into the

tissue. The UOT signal will be registered alongside with the US images. The US images provide an anatomical tissue reference (e.g. skin, subcutaneous fat, muscle, breast parenchyma, vessels, bones) as well as the depth (in mm from the skin). Each measurement will take approximately up to 10 minutes, most likely shorter. The measurement time is dependent on the type of tissue and thickness. Multiple measurements will be performed on arms and legs and breasts, according to a scheme (Bilaga 4), during a maximum of 60 minutes in total per subject. There will be continuous monitoring during the procedures. Researchers will be present throughout the procedure to monitor the reactions of the research object and promptly intervene if any adverse effects arise. Before the subject leaves, visual inspection of the skin in contact with the probes will be performed. 24h after finalization of the measurements, the performing researcher will follow up any adverse effects through a mobile text message 24h after the end of the measurements. If any adverse effects apply that are judged in need of surveillance or treatment as deemed by the PI and sponsor with medical profession, the subject will be recommended to consult regular health care.

Each subject's participation is expected to take about 2h during Day 1 and a short follow-up 24h later. The estimated enrolment period is six months up to one year.

6.7. End of the clinical investigation

The clinical investigation ends when the last subject has completed the last follow-up. The sponsor will notify the Swedish Medical Products Agency within 15 days after the end of the clinical investigation and send the clinical investigation report within 1 year after the end of the clinical investigation including an easily understandable summary.

6.8. Biological sampling procedure (if applicable)

Not applicable.

6.9. Monitoring plan

The clinical investigation will be monitored by an independent monitor before the clinical investigation begins, during the clinical investigation conduct, and after the clinical investigation has been completed, so as to ensure that the clinical investigation is carried out according to the CIP and that data is collected, documented, and reported according to SS-EN ISO 14155:2020 and applicable ethical and regulatory requirements. Monitoring is performed as per the investigation's monitoring plan and is intended to ensure that the subject's rights, safety, and well-being are met as well as data in the database are complete, correct, and consistent with the source data. All study documents will be kept in an investigator site file. Source data will be made available to the study monitor, by access to the written study protocols for each study individual. Any other data needed for verification will be made available, such as measurement history registered on laptops used.

7. Statistical considerations

7.1. Analysis population

Exploratory study on healthy volunteers why the analyses at this stage do not aim to be applicable to intention-to-treat or per protocol analyses.

7.2. Descriptive statistics

Presentation of the study population will include the variables in the measurement scheme (bilaga 4):
Age (years), Body mass index (weight (kg)/(height (m))^2, skin tone according to Fitzpatrick scale (1-6), fat percent on skin (%) arm and leg.
UOT signal in relation to depth in mm at the different measurement points.
Calculations of oxygenation (%) in relation to different tissue types.

7.3. Analytical procedures

Since the project is highly exploratory, mainly descriptive data will be used including e.g. range, mean, median, and standard deviation. Potential differences between groups will be described and analyzed with appropriate statistics. Tissue contrast will be evaluated using the UOT signal contrast-to-noise ratio at different depths and compared with the co-registered US signal as performed in our previous work (4, 36, 37).

To evaluate the technique's ability to detect small signal changes, we will estimate the contrast-to-noise ratio (CNR) of each UOT image as defined by D. Dieckens et al. (46):

$$CNR = \frac{|\mu_{signal} - \mu_{noise}|}{\sigma_{noise}}. \qquad (1)$$

Here, μ_{signal} and μ_{noise} are the mean (average) pixel values from two regions of interest (ROI) of the UOT image, one for signal and one for noise. The σ_{noise} is the standard deviation of the pixels in the noise region only. To distinguish slight contrast variations between the inclusion and the surrounding tissue, the CNR should be above 1.

7.4. Sample size calculation

The project is at this stage highly exploratory since this is the first test in humans of the UOT technique. The proposed project parts should hence be considered as proof-of-concept studies. The number of healthy volunteers proposed are planned to ensure representation of variety of different tissue characteristics. We plan to include 30 healthy volunteers, both males and females in about 50/50% proportions for arm and leg measurements and 30 females for breast tissue measurements, so in total 60 persons. The results will guide design and power considerations of future clinical trials with the technique.

8. Data management and protection

Subjects who participate in the clinical investigation are coded with a specific clinical investigation identification number (Study ID). All subjects are registered in a subject identification list (subject enrolment and identification list) that connects the subject's name and personal number with a clinical investigation identification number. This list will be retained by the principal investigator in a secure location. All data will be registered, managed, and safely stored in a manner that enables correct reporting, interpretation, and verification.

8.1. Case Report Form

Images recorded during the measurements will be stored on a local computer. The research subject data collection will be performed according to a form (Bilaga 4) and will be filled out on paper. These forms will be kept in secure locations and will only be accessible to authorized personnel involved in the study. Collected data (images and meta-data) will be processed in a database in accordance with applicable laws and ethical guidelines to ensure the integrity and confidentiality of research subjects. Personal data will be pseudonymized, i.e. replaced by a Study ID and the key will be stored securely. The original data will only be accessible by the investigators by personal login on both the computer and the database.

8.2. Data cleaning and database lock

The database will be reviewed and verified against the source data continuously to resolve any data queries. This exploratory proof-of-concept study does not aim for clinical implementation, and a formal database lock is not fully applicable. Nevertheless, the database will be considered finalized once all data points have been entered, checked and monitored accordingly.

8.3. Archiving

The PI and sponsor will maintain the essential clinical investigation documents in the investigation site files archive and sponsor files archive, respectively. The sponsor shall keep all documentation and data for at least 10 years after the clinical investigation has ended. The PI will archive all local investigation documentation for at least 10 years or as long as stipulated by the local institution.

8.4. Data protection

If any part of the data is handled by any other organization, inside or outside the European Union, appropriate agreements and/or other documentation will be established, to ensure that the data processing is performed in accordance with the provisions of the General Data Protection Regulation (EU ordinance 2016/679, GDPR) and other relevant legislation, before any data transfer takes place.

The content of the informed consent form shall comply with relevant integrity and data protection legislation. In the subject information and the informed consent form, the subject will be given complete information about how collection, use and publication of their clinical investigation data will take place. The subject information and the informed consent form will

explain how clinical investigation data are stored to maintain confidentiality in accordance with national data legislation.

Images recorded during the measurements will be stored on a local computer. The research subject data collection will be performed according to a form (Bilaga 4) and will be filled out on paper. These forms will be kept in secure locations at the study site and will only be accessible to authorized personnel involved in the study. Collected data (images and meta-data) will be processed in a database in accordance with applicable laws and ethical guidelines to ensure the integrity and confidentiality of research subjects. Personal data will be pseudonymized, i.e. replaced by a Study ID and the key will be stored securely. The original data will only be accessible by the investigators by personal login on both the computer and the database.

Access to data will be handled strictly by authorized persons. Only those individuals necessary to conduct the study will be given access to the collected information. These people will be well informed about the GDPR requirements and the ethical guidelines for data protection. By restricting access to authorized personnel only, the risk of accidental or unauthorized dissemination of sensitive information is minimized. This ensures the research subjects' integrity and trust in the study.

The informed consent form will also explain that for verification of the data, authorized representatives of the sponsor, as well as relevant authority, may require access to parts study records that are relevant to the clinical investigation, including the subject’s medical history.

In case of a suspected data security breach, in order to mitigate the possible adverse effects, we follow Lund University’s regulations for reporting and mitigation: https://www.staff.lu.se/support-and-tools/personal-data-and-data-protection/how-manage-security-breach-involving-personal-data

9. Amendments to the CIP

Amendments to the CIP will be agreed upon between the principle investigator (in this case also the sponsor) and the involved researchers. Substantial modifications must be approved by the Swedish Ethical Review Authority and/or the Swedish Medical Products Agency (as applicable) before implementation.

10. Deviations from the CIP

The investigator is not allowed to deviate from the CIP except if it is for the protection of the subject´s rights, safety, or well-being under emergency circumstances. All such deviations shall be documented and reported by the sponsor/investigator to the Swedish Medical Products Agency and/or the Swedish Ethical Review Authority (as applicable) as soon as possible.

All deviations shall be documented with an explanation and reported to the sponsor. Deviations will be reviewed by the sponsor and reported to the appropriate regulatory bodies as required.

11. Device traceability and accountability

Stefan Kröll, Professor at the Division of Atomic Physics, Department of Physics, Lund University is responsible for the research device, Mk1, to be used in the project (bilaga 6c). He controls the access to the device and secures its use in the clinical investigation. In case of replacement of malfunctioning device parts, this will be recorded in the accountability log in the investigator site file. The device remains at Lund University after the study.

12. Statements of compliance

12.1. Compliance to the investigational plan, good clinical practice, and regulations

The clinical investigation will be conducted in accordance with the clinical investigation plan, the ethical principles of the Declaration of Helsinki, the principles of SS-EN ISO 14155:2020 and current national and international regulations governing this clinical investigation. This is to ensure the safety and integrity of the subjects as well as the quality of the data collected.

12.2. Ethical review of the clinical investigation

The clinical investigation will commence when written approval/favorable opinion from the Swedish Ethical Review Authority has been received and confirmation of validity has been received from the Swedish Medical Products Agency.

The final version of the informed consent form and other information provided to subjects, must be approved or given a written positive opinion by the Swedish Ethical Review Authority and/or the Swedish Medical Products Agency *(as applicable)*. The Swedish Ethical Review Authority and the Swedish Medical Products Agency must be informed of any changes in the CIP in accordance with the current requirements.

12.3. Insurance

The Department of Translational Medicine at Lund University will get a collective insurance that covers the individual research subjects via the Kammarkollegiet for Special personal injury insurance (Kammarkollegiet för Särskilt personskadeskydd. Terms and conditions for the insurance can be found here:https://www.medarbetarwebben.lu.se/anstallning/forsakringar/forsakringar-gaster-och-forsokspersoner

https://www.kammarkollegiet.se/vara-tjanster/forsakring-och-riskhantering/forsakringar-for-personskador/sarskilt-personskadeskydd

The insurance will be set in place when written approval/favorable opinion from the Swedish Ethical Review Authority has been received and confirmation of validity has been received from the Swedish Medical Products Agency and before the start of the study. It will cover the 40 research persons during the procedures and up to 24h (including the last follow-up). The insurance is deemed suitable with regard to potential risks. The terms and conditions of the insurance can be found in Bilaga 5.

13. Informed consent process

13.1. General process for informed consent

The principal investigator shall ensure that the subject is given full and adequate oral and written information about the clinical investigation, its purpose, any risks and benefits as well as inclusion and exclusion criteria. Subjects must also be informed that they are free to discontinue their participation in the clinical investigation at any time without having to provide a reason. Subjects shall be given the opportunity to ask questions and be allowed time to consider the provided information and participation in the clinical investigation. If the person chooses to participate, both the subject and the investigator shall sign the informed consent form. A copy of the subject information as well as a copy of the informed consent form shall be provided to the subject. The subject's signed and dated informed consent must be obtained before performing any activity specific to the clinical investigation. The process shall be documented in the subject's source documents and the signed informed consents shall be maintained with the essential documents. If new information becomes available that can significantly affect a subject's future health and medical care, that information shall be provided to the affected subject(s) in written form. If new information is added to the clinical investigation, the subject has the right to reconsider whether he/she will continue their participation.

13.2. Informed consent process for vulnerable populations

Not applicable.

14. Adverse events, adverse device effects and device deficiencies

14.1. Definitions

14.1.1. Adverse Event

An Adverse Event (AE) is untoward medical occurrence, unintended disease or injury or any untoward clinical signs, including an abnormal laboratory finding, in subjects, users or other persons, in the context of a clinical investigation, whether or not related to the investigational device.

This definition includes events that are anticipated as well as unanticipated events

This definition includes events occurring in the context of a clinical investigation related to the investigational device, the comparator or the procedures involved.

14.1.2. Adverse Device Effect

An Adverse Device Effect (ADE) is any AE related to the use of an investigational medical device.

This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.

This definition includes any event resulting from user error or from intentional misuse of the investigational medical device.

14.1.3. Serious Adverse Event

A Serious Adverse Event (SAE) is any AE that led to any of the following:

a) death,

b) serious deterioration in the health of the subject, that resulted in any of the following:

   i. life-threatening illness or injury,

   ii. permanent impairment of a body structure or a body function,

   iii. hospitalization or prolongation of patient hospitalization,

   iv. medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,

   v. chronic disease,

c) fetal distress, fetal death or a congenital physical or mental impairment or birth defect

14.1.4. Serious Adverse Device Effect

A Serious Adverse Device Effect (SADE) is an ADE that has resulted in any of the consequences characteristic of a serious adverse event.

SAEs related to procedures imposed by the clinical investigation plan but not with the use of the device shall not be considered Serious Adverse Device Effects.

14.1.5. Unanticipated Serious Adverse Device Effect

An Unanticipated SADE is an effect which by its nature, incidence, severity or outcome has not been identified in the current risk assessment. Procedures associated with the use of a device shall be addressed in the risk assessment, which makes it possible to determine whether the procedure related SAEs are Unanticipated Serious Adverse Device Effect or not. SAEs related to procedures imposed by the clinical investigation plan but not with the use of the device shall not be considered Serious Adverse Device Effects.

For the anticipated adverse device effects, see section 4.2 above.

14.1.6. Device Deficiency

A Device Deficiency (DD) is any inadequacy in the identity, quality, durability, reliability, safety or performance of an investigational device, including malfunction, use errors or inadequacy in information supplied by the manufacturer.

14.2. Recording and Reporting

14.2.1. Recording

The principal investigator or an authorized designee will record:

- all AEs except the events specified not to be recorded; discomfort related to positioning during measurement, since the time frame for the examination will not be representative for a future clinical device that will perform the measurements much quicker than the current investigational device.
- all SAEs;
- all DDs;
- any new findings in relation to any of the above-mentioned events.

14.2.2. Reporting

The sponsor/investigator will report to the Swedish Medical Products Agency all of the following reportable events:

- any SAE that has a causal relationship with the investigational device or the investigation procedure, or where such causal relationship is reasonably possible;
- any DD that might have led to a SAE if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate; and
- any new findings in relation to any event referred to above.

Reporting by the sponsor will be done by filling out the “Summary Reporting Form” (MDCG 2020-10/2). The form will be filled in/updated for each reportable event or for new findings/updates to already reported events. The form will be transmitted to the Swedish Medical Products Agency. For events that indicate an imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for other patients/subjects, users or other persons or a new finding to it will be reported immediately, but not later than 2 calendar days after awareness by sponsor of a new reportable event or of new information in relation with an already reported event. Any other reportable events or a new finding/update to it will be reported immediately, but not later than 7 calendar days following the date of awareness by the sponsor of the new reportable event or of new information in relation with an already reported event.

14.2.3. Assessment of Causality

The relationship between each adverse event and the investigational device, and the investigation procedure will be assessed and recorded by the investigator/sponsor. For assessment of causality, the IB and the risk analysis report will be consulted. The sponsor/investigator will distinguish between SAEs related to the investigational device and those related to the procedures, relatedness to both is possible.

Each SAE will be classified according to four different levels of causality:

1. Not related

Relationship to the device, comparator or procedures can be excluded when:
- the event has no temporal relationship with the use of the investigational device, or the procedures related to application of the investigational device;
- the SAE does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible;
- the discontinuation of medical device application or the reduction of the level of activation/exposure - when clinically feasible - and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious adverse event;
- the event involves a body-site or an organ that cannot be affected by the device or procedure;
- the SAE can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors);
- the event does not depend on a false result given by the investigational device used for diagnosis, when applicable;

In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the SAE.

2. Possible
The relationship with the use of the investigational device or comparator, or the relationship with procedures, is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases where relatedness cannot be assessed, or no information has been obtained shall also be classified as possible.

3. Probable
The relationship with the use of the investigational device or comparator, or the relationship with procedures, seems relevant and/or the event cannot be reasonably explained by another cause.

4. Causal relationship
The SAE is associated with the investigational device, comparator or with procedures beyond reasonable doubt when:
- the event is a known side effect of the product category the device belongs to or of similar devices and procedures;
- the event has a temporal relationship with investigational device use/application or procedures;
- the event involves a body-site or organ that
    - the investigational device or procedures are applied to;
    - the investigational device or procedures have an effect on;
- the serious adverse event follows a known response pattern to the medical device (if the response pattern is previously known);
- the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the SAE (when clinically feasible);
- other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
- harm to the subject is due to error in use;

– the event depends on a false result given by the investigational device used for diagnosis, when applicable.

In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the SAE.

14.3. List of foreseeable Adverse events

Please refer to the Investigator Brochure.

Below is a list of potential SAEs or SADEs.

Anticipated SAE/SADE	Related to device or procedure	Probability of occurrence
Electric shock due to electrical part failure - Electrocution	Device	Very unlikely
Unintended laser emission from the device - Eye injury	Device or procedure	Very unlikely
Respiratory issue due to the helium leakage - Asphyxiation	Device	Very unlikely
High mechanical index of Ultrasound - Skin or tissue damage or blood vessel rupture	Device	Very unlikely
High thermal index of Ultrasound - Skin or tissue damage	Device	Very unlikely
Skin burns due to simultaneous use of laser and ultrasound - Skin burns, pain, and tissue damage	Device	Very unlikely

The probability of occurrence of these events was qualitatively estimated by the group based on the combined experiences of the members of the research group. It is based on the risks related to these events that have been mitigated as described in the risk analysis.

14.4. Data Monitoring Committee

Not applicable.

15. Premature termination of the clinical investigation

The sponsor may suspend or prematurely terminate the entire clinical investigation for significant and documented reasons. The Swedish Medical Products Agency may suspend or prematurely terminate the clinical investigation at the investigation site.

If suspicion of an unacceptable risk to subjects arises during the clinical investigation, or when so instructed by the Medical Products Agency, the sponsor will suspend the clinical investigation while the risk is assessed. The sponsor will terminate the clinical investigation if an unacceptable risk is confirmed. The sponsor will inform all investigators.

The sponsor shall consider terminating or suspending the participation of a particular investigation site or investigator in the clinical investigation if monitoring or auditing identifies serious or repeated deviations on the part of an investigator. If the suspension or premature termination was in the interest of safety, the sponsor shall inform all other principal investigators.

If, in the opinion of the investigator, the clinical observations in the clinical investigation suggest that it may be unsafe to continue the investigation at the site, the investigator may terminate participation in the investigation after consultation with the sponsor. A written statement fully documenting the reasons for such termination will be provided to the sponsor. If the clinical investigation is prematurely terminated, the investigators shall promptly inform the subjects and take necessary steps to finalize their engagement in the clinical investigation. All relevant investigation materials must be collected, and accountability completed.

If the clinical investigation is interrupted or terminated prematurely, the sponsor will report to the Medical Products Agency within 15 days together with a justification. If the sponsor has temporarily halted or prematurely terminated the clinical investigation on safety grounds, the Medical Products Agency will be informed within 24 hours. A clinical investigation report will be prepared within three months of the early termination or temporary halt, irrespective of the results. In the event that the clinical investigation is restarted within three months of the temporary halt, the sponsor does not have to submit a clinical investigation report until the clinical investigation has been completed.

The final clinical investigation report shall include details with respect to the temporary halt.

Since the follow-up is 24h, there are no specific procedures for a temporary halt or early termination of the investigation.

16. Publication policy

The study will be registered in a publicly available database (clinicaltrials.gov). The clinical investigation will be registered in a publicly accessible database before the start of recruitment activities and the content will be updated throughout the conduct of the clinical investigation and the results entered at completion of the clinical investigation. The results of the study will

be made publicly available through conference abstracts and scientific reports published in peer-reviewed scientific journals during the upcoming 1-5 years after study completion. The study is an academic project, and the sponsor is the principal investigator of the study and will be responsible for the scientific reports of the results from the study. Co-authorship will be applied according to the regulations of the International Committee of Medical Journal Editors, e.g. the so-called Vancouver principles.

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