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Public Final Report

MIRACLE Deliverable D8.9

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1. Contents

List of Abbreviations

AgX	silver halide
ATR	attenuated total reflection
CAD	computer-aided design
CNC	computerized numerical control
CVD	Chemical Vapor Deposition
DD	Digital Densitometry
DFB	Distributed Feedback Laser
Di-ATR	Diamond ATR
EEL	Edge-Emitting Laser
f-SMA	fiber sub-miniature-A
FTIR	Fourier Transform Infrared
GUI	Graphical user interface
ICRS	International Cartilage Regeneration & Joint Preservation Society
iHWG	substrate-integrated hollow waveguides
IR	Infrared
IU	User interface
MBE	Molecular Beam Epitaxy
MCT	mercury cadmium telluride (detector)
ATR	attenuated total reflection
MIR-ATR	Mid-Infrared Attenuated Total Reflection
MSC	Multivariate Signal Correction
OA	Osteoarthritis
OAP	off-axis parabolic
OARSI	Osteoarthritis Research Society International
OD	Outer Diameter
PIR	Polycrystalline Infrared
PLM	Polarized Light Microscopy
PLS-DA	Partial least squares discriminant analysis
PTOA	Post traumatic Osteoarthritis
QCL	quantum cascade laser
RF	Random forest
RIE	reactive ion etching
SaaS	Software as a service
SMSR	Side Mode Suppression Ratio
SNR	Signal-to-Noise Ratio
SPLS-DA	Sparse PLS-DA
SVM-DA	Support Vector Machine Discriminant Analysis
URL	Uniform resource locators

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2. Summary

MIRACLE project main goal is to develop, validate and bring close to market the first Mid Infrared (MIR)-based arthroscopy system for the quantitative assessment of articular cartilage. This is intended to provide precise and accurate measurements of the status of articular cartilage, enabling early diagnosis of degenerative joint diseases such as osteoarthritis (OA). The envisioned device is intended for use during a minimally invasive surgery (arthroscopy), providing orthopaedic surgeons a decision support aid to decide on the most adequate therapeutic route for individual patients, striving towards personalized medicine. Surgeons and practitioners working in orthopaedics will have access to a system that will allow them to pinpoint specific areas of knee cartilage that are degenerated or damaged and based on such information make an informed decision on the best course of treatment for a specific patient.

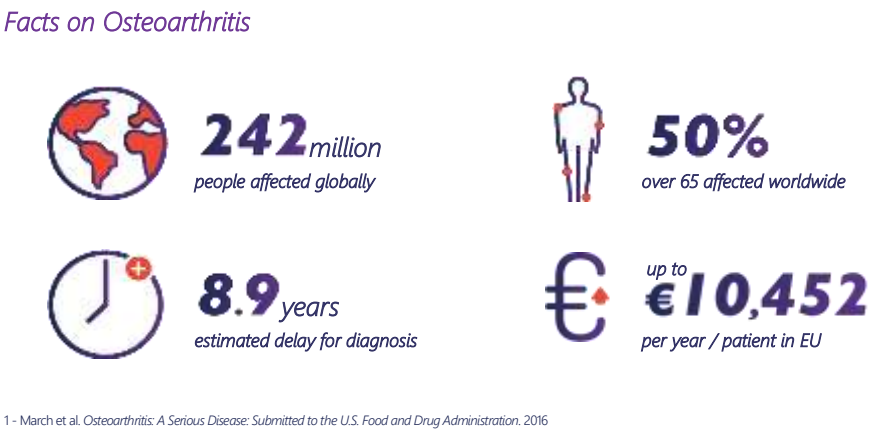


Figure 1 Facts on osteoarthritis

Currently, the surgeon's decision-making is based on visual inspection and manual probing of the cartilage tissue which is highly subjective and of poor repeatability. Untreated or not-correctly treated joint injury will most likely progress towards OA, which will lead to joint pain, movement limitation, joint failure, and ultimately disability and joint replacement. OA constitutes a major challenge for the health systems and affects 242 million people globally. Moreover, OA is highly prevalent in Europe with an estimated 19.7-42.3% in the elderly population.

Figure 2 the MIRACLE solution

The MIRACLE concept is to access the articular cartilage and predict the biomechanical properties which precedes OA based on the biochemical constitution of the cartilage. As the first medical device to provide quantitative examination of cartilage tissue in real-time, it is expected that MIRACLE arthroscopy system will bring innovation to the arthroscopy market valued at $4 billion in 2015. Furthermore, it is expected that MIRACLE will bring to the market three novel photonics components:

- Tailored quantum cascade lasers (QCLs) for biodiagnostics.
- Integrated beam combiner for efficient radiation coupling.
- MIR sensing probe.

These components were assembled into the first MIR arthroscopy system prototype. The MIRACLE project also addresses the data processing and analysis to enable real-time grading of the cartilage tissue. To achieve these goals, MIRACLE has an active medical team participating in each step of the device development ensuring that the clinical needs are achieved according to the surgeon's expectations. It is intended that the medical device will be used during arthroscopy without interfering with the current surgery protocols ensuring a smooth transition from Research & Development (R&D) to clinical use.

These components will be integrated in a medical device to be placed in the arthroscopy market (valued at $4 billion in 2015). In addition to add value to the European medical equipment industry, MIRACLE strives towards cost reduction of OA patients (currently costs/patient/year €10,452) contributing to more affordable public health care and promoting wellbeing in the European ageing population.

The novel arthroscopy device is thus expected to lead to improved clinical decision-making and consequently to improved patient recovery and well-being, as well as to cost savings due to recovery times. The arthroscopy device can be further adapted for use in other joints (*e.g.*, ankle, shoulder, elbow) and for veterinarian use, with focus in horses and camels, expanding the market share available and opening new markets for the industrial partners involved.

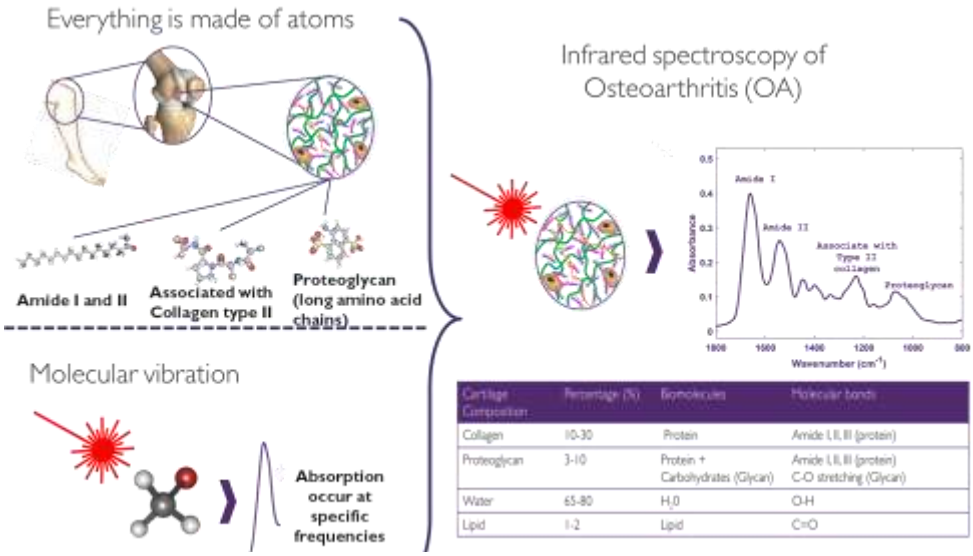


Figure 3 the MIRACLE proposition

3. The MIRACLE Project in a Nutshell

3.1. Project Partners

Figure 4 MIRACLE partners across EU

UOULU	Oulu University	Oulu, Finland
UULM	Ulm University	Ulm, Germany
UEF	University of Eastern Finland	Kuopio, Finland
NMBU	Norway's environment and life science university	Ås, Norway
KUH	Kuopio University hospital	Kuopio, Finland
UU	Utrecht university	Utrecht, the Netherlands
NP	Nanoplus	Gerbrunn, Germany
OP	Optoprecision	Bremen, Germany
IRIS	Innovacio I Recerca industrial I Sostenible sl	Castelldefels, Spain
AP	Art Photonics	Berlin, Germany
INEB	Instituti nacional de Engenharia biomedica	Porto, Portugal
UNE	Asociación Española de Normalización	Madrid, Spain
PF	Photonics Finland	Joensuu, Finland
UCP	Universidade Católica Portuguesa	Porto, Portugal

3.2. Overview

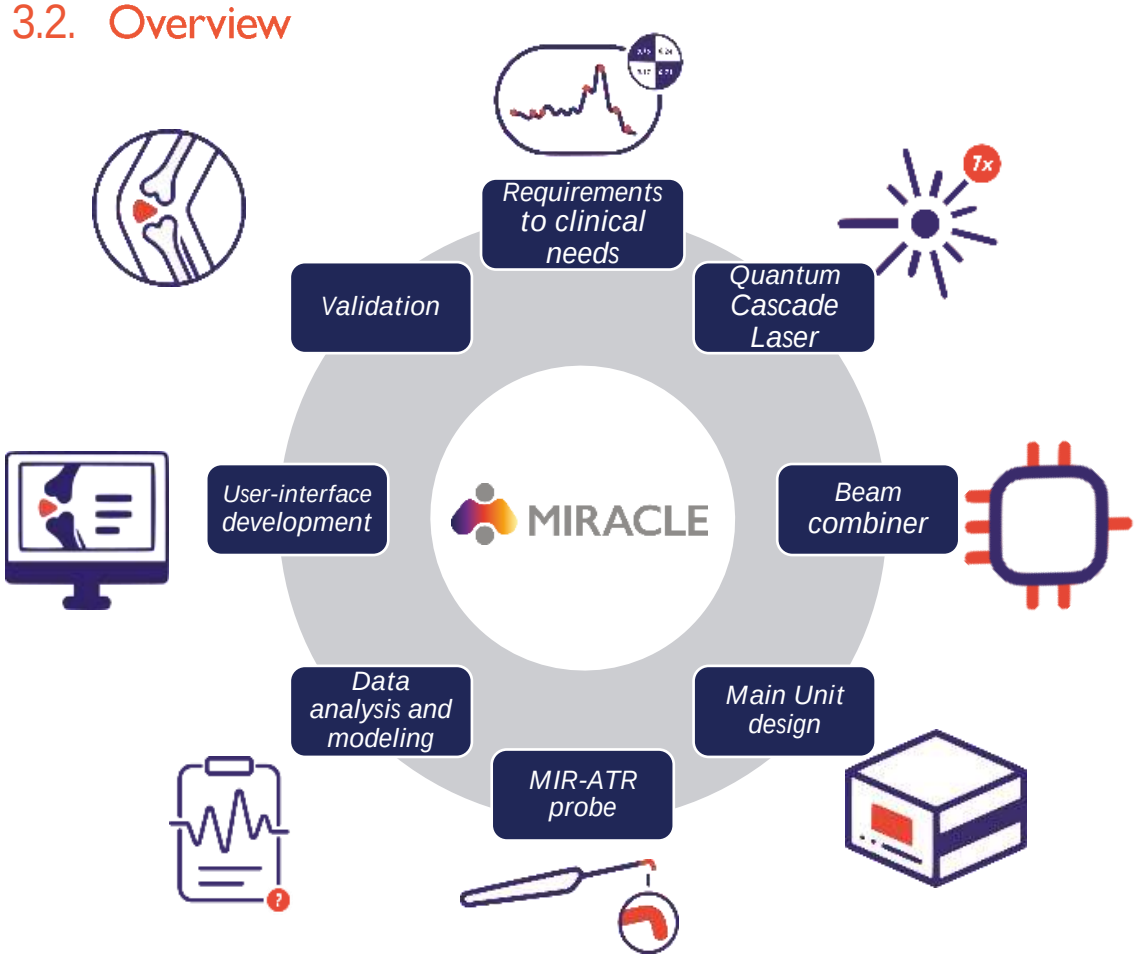


Figure 5 MIRACLE in a nutshell

The project can be divided in a number of tasks:

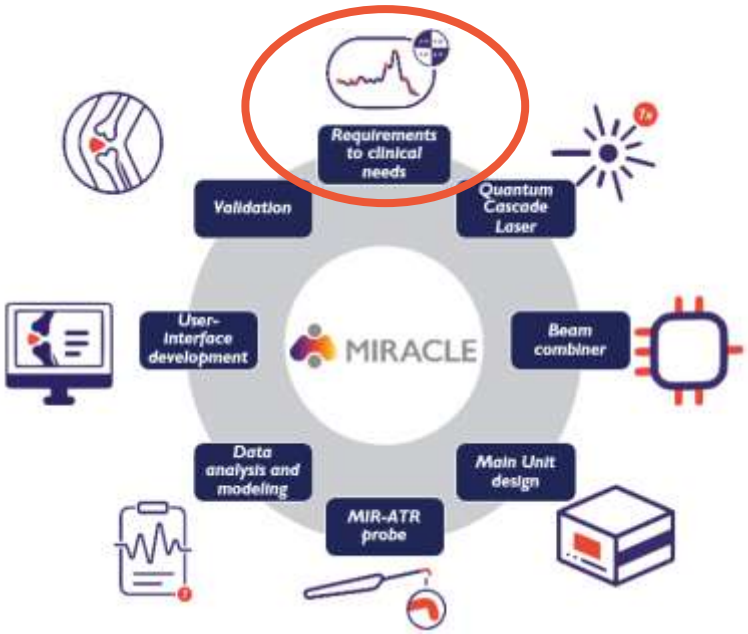
- Requirements for clinical needs
- Quantum Cascade Lasers
- Beam combiners
- Main unit
- MIR Attenuated Total Reflection (ATR) probe
- Data analysis and modelling
- User Interface Development
- Validation

Additionally, the following tasks were or major importance during the project's entire duration:

- Project management
- Project communication and dissemination
- Business and exploitation plans

4. MIRACLE Tasks and Components

4.1. Requirements to Clinical Needs



Specifications and requirements for the MIR-ATR arthroscopy probe

The starting point of the project consisted in identification the clinical requirements and establishing the technical specifications of the MIR arthroscopic probe. Particular emphasis was given to the technical specifications, such as: required sizes and other external measurements of the probe, specifications related to the wavelength and laser, specifications for the data analysis and software. Following discussions regarding ow to overcome current technical limitations, detailed technical specifications were defined, including the basic starting design for the prototype development. A summary of the specifications and requirements for the MIR-ATR arthroscopy system is showed in Table 1

Table 1 Summary of the defined specifications and requirements for product development

Specifications and requirements	MIRACLE approach
'should be small enough and comparable to standard probe used in arthroscopy'	Dummy probe was designed with **3mm** diameter.
'withstand sufficient force'	Probe designed to support maximal applied force on the probe 50 N (overestimation).
'hook-probe similar to standard probe'	MIR-ATR designed with hook-shape.
'built with biocompatible materials'	Medical grade stainless steel custom 455.
Light source	QCLs with beam combiner
Wavelength	Select 7 wavenumbers according to clinical relevance.

'Probing should not require staying too long at the same place as the friction coefficient of the cartilage is extremely low. (probing should be fast in order not to prolong surgery time)'	Aiming at 2-3 seconds/spot, starting with 1min/spot.
Contact force during the measurement	Below 1N to prevent iatrogenic damage
Disposable or reusable	Reusable.
Method of sterilization	Autoclave (137°C, high pressure).
Penetration depth of measurement	<10 μm, since MIR is a surface measurement.
Detector	MCT or pyroelectric.

Ethics

Ethical evaluation is a continuous process that considers the prevalent morals, values, and behavioral models of the society. Each research study creates its own ethical rules and regulations that are the basis for their research; these combine knowledge, experience, and commitment to ethically acceptable goals. The ethics aspects in MIRACLE project relate to the execution of the clinical study and to the assessment of clinical impacts.

During the project all documentations required for human and equine subjects were prepared, including, but not limited to ethical approval, details on recruitment, criteria for inclusion, informed consent, and data protection. These documents ensure that:

- All regulations and measures of confidentiality and data security for human cell/tissue and human cadavers are strictly enforced according to UEF guidelines, national legislation and in accordance with the Declaration of Helsinki.
- All animal experiments will be reviewed and authorized by the local animal experimentation committees.
- All experiments will be performed by authorized and specifically trained personnel and will respect the rules of national ethical and medico-ethical committees.

To ensure that the all the project activities and deliverables comply with the ethical principles and guidelines of H2020 Program and relevant national, EU and international legislation, an Ethics Committee has been appointed for the whole duration of the project.

Clinical protocol development

Clinical Protocol confirms that ethical principles and good clinical practices are conducted properly during validation in clinical settings involving human tissues samples and human subjects.

The relevant national and EU guidelines adopted during MIRACLE validation in clinical settings of the novel diagnostic system via environment testing in *ex-vivo* human cadaver knees, and *in-vivo* human patients are used for clinical protocol preparation. In addition, the most relevant European and International Standardization Technical Committees, European and International published Standards and Regulations have been applied to the MIRACLE project.

4.2. Quantum Cascade Laser

The MIRACLE team identified seven wavelengths that could be used to distinguish between damaged and healthy cartilage. These wavelengths are between 5-12 µm, so lay in the mid-infrared (MIR) region. Quantum cascade lasers (QCL) can cover this range of wavelengths.

Fabrication of such lasers is done in three steps: epitaxial growth, chip processing, and packaging. NP has facilities and instruments for all the steps within the company. The material of choice for MIR lasers is InGaAs/InAlAs/InP, which can cover all the selected wavelengths.

Four different layering designs were used to cover all seven wavelengths (Figure 6). Molecular beam epitaxy (MBE) is a technique commonly used to fabricate QCL. NP has a dedicated MBE facility for the fabrication of QCL. After the epitaxial stage, the wafer should be processed for laser fabrication. As the MIRACLE project requires a very narrow band laser a special processing technique must be used for the manufacturing of the laser chips. We used distributed feedback laser (DFB) technique to achieve an extremely narrow banded laser with a high side mode suppression ratio (SMSR). In this technique (Figure 6 Schematic illustration of the QCL DFB laser design.) a Bragg reflector is placed on the top of the active region. The wavelength and its width can be controlled by the periodicity and the effective refractive index of the grating. The produced lasers can be tuned using temperature, which is very useful for spectroscopic application.

The processing of such samples is complicated and one needs highly accurate instruments such as electron beam lithography, optical lithography, chemical/ion etching, mirroring, etc. The sample is inspected in every step and tested after mirroring in bar level (there are several lasers on one bar). If the process ran as planned and the bar level tests show the lasing within the required spec the laser moved to the next step which is the packaging.

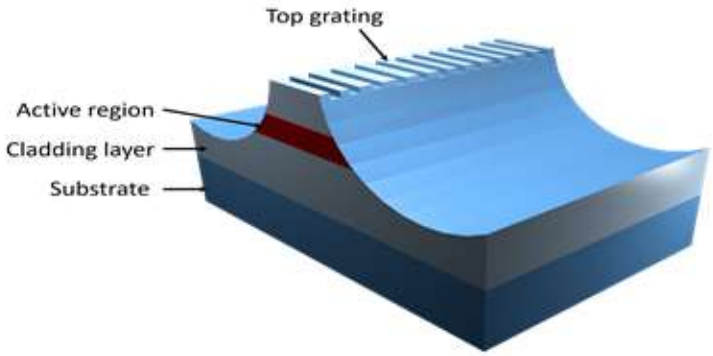


Figure 6 Schematic illustration of the QCL DFB laser design.

For the MIRACLE project, the lasers are mounted in the TO3 package with the ability to control the temperature down to 15ºC or even lower (Figure 7). Due to the structure of edge-emitting laser (EEL), the laser light is highly divergent, so one needs lenses to shape the beam with very low divergent. For each wavelength aspherical lens with low absorption material and coated with anti-reflective layers were used. After choosing the right lenses the lasers are collimated and finally capped to protect them from environmental elements such as humidity and dust.

Figure 7 TO3 packaging

After packaging is completed the final characterization of the laser will be carried out. The lasers are tested for the SMSR, tuneability (Figure 8), the laser threshold, and efficiency (Figure 9). NP has successfully developed and delivered the series of lasers within provided specification.

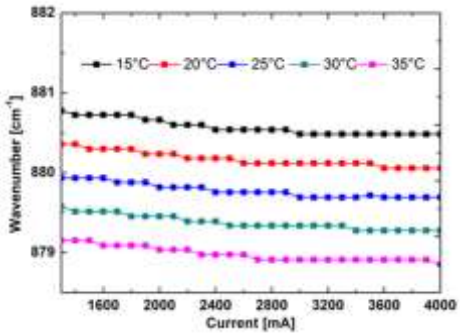


Figure 8 Wavenumber tuning characteristic of the collimated laser device at operation temperatures between 15 - 35°C set by TEC. The laser is driven in pulsed mode at a repetition rate of 100 kHz and a pulse width of 150 ns.

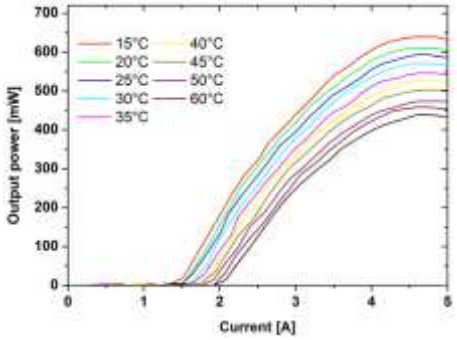


Figure 9 P-I characteristic of the collimated laser device at operation temperatures between 15 – 60 °C set by TEC. The laser is driven in pulsed mode at a repetition rate of 1 kHz and a pulse width of 150 ns.

To use the laser safely to the patient body, one needs to couple the laser to a fiber. A stable and reliable fiber coupling is very critical for the applicability of the laser device. Traditionally MIR lasers are coupled to the fiber using a concaved mirror, which is bulky, mechanically very sensitive, and unstable. Therefore, Nanoplus developed a lens-on-cap adaptor (Figure 10). This concept was used for all the lasers and tested successfully. The lens-on-cap adaptor construct is very user-friendly, and the tests show a stable and reliable coupling. It is already available as a standard product in Nanoplus.

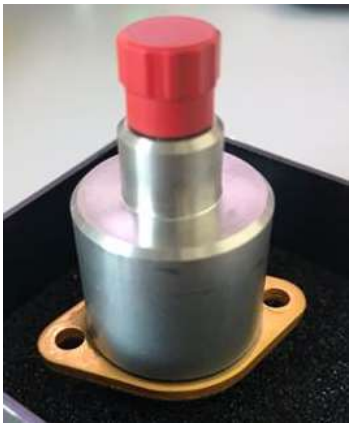


Figure 10 Lens-on-Cap adaptor

4.3. Beam Combiner

Within the MIRACLE project, seven individual QCLs are developed by NP. These QCLs are designed to emit at distinct and individual wavelengths, which are clinically relevant. The beam combiners are designed to receive light from several input-ports and combine them into a single output-port. Hence, the output-port comprises multispectral light which is crucial for the MIR-ATR based device as developed within the scope of the MIRACLE project. This multispectral port is then used to be coupled to the final MIR-ATR arthroscopy probe. Consequently, multispectral QCL light can be utilized to gather spectral information about the probed cartilage. The beam combiner is therefore an essential part of the device that significantly affects the performance of the ATR probe. In addition, the beam combiners have been continuously miniaturized to enable a compact device design as well. Three different concepts of beam combiners were simultaneously adopted:

- Hollow waveguide-based beam combiner
- Fiber-based beam combiner
- On-chip dielectric waveguide-based beam combiner

Hollow waveguide-based beam combiner

Hollow waveguides represent waveguides based on guiding light via highly reflective metal mirrors. Based on fundamental concepts pioneered at IABC/UULM, substrate-integrated hollow waveguides (iHWGs) were utilized for this development (iHWG-Beam). The reflective elements, a.k.a. side walls of the waveguide slot, are milled from solid aluminum due to robustness, good manufacturing properties and material inherent high MIR reflectivity, which results in good radiation guiding properties. CAD-based

design and CNC based fabrication led to high accuracy in light pipe design, high freedom of design as well as high surface quality. Figure 11 shows the newest version of the iHWG beam that can be installed in the MIRACLE device with satisfactory performance. By using PIR-AgX fibers, the distinct laser radiations are directed to the beam combiner, the resulting multispectral light can be coupled directly to the ATR probe via f-SMA connection.

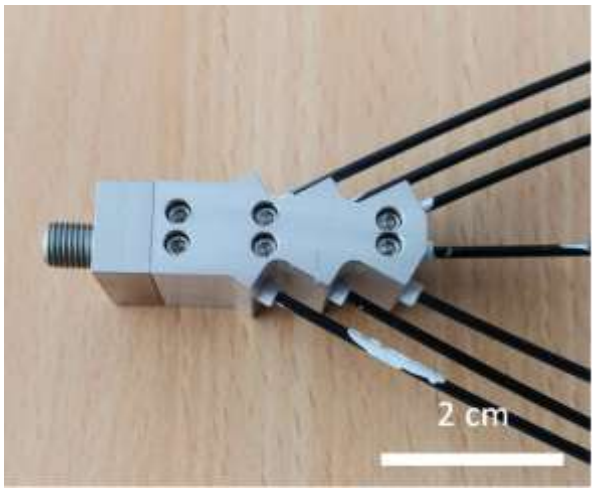


Figure 11 7th generation of iHWG-Beam

Fiber-based beam combiner

An alternative solution for coupling the 7 QCLs into the ATR arthroscopy probe was made with an PIR AgX fiber combiner by AP (Figure 12). This combiner consists of 7 PIR AgX fibers built as a fan-out bundle. The fibers were arranged together in a hexagonal shape (6-around-1) and had on all ends SMA connectors to enable direct mounts onto corresponding QCLs and further coupling optics. The length of each leg was accurately calculated based on the main units CAD model. Due to the flexibility of the fibers, it is possible to integrate the fibers extremely precisely, considering the ideal bending radii. In addition, PIR AgX fibers have a high MIR transparency, which is why good performances could also be achieved. The fiber-based beam combiner showed a significant stronger performance than the iHWG combiner, therefore it was also used during the human cartilage measurements for the model development.

Figure 12 PIR AgX fiber combiner

On-chip dielectric waveguide-based beam combiner

The on-chip dielectric waveguide combiner strategy is based on structuring via reactive ion etching (RIE) for realizing the complex geometrical structure of the on-chip iBEAM based on GaAs/AlGaAs. To advance progress for on-chip iBEAM waveguide design strategies, NP and UULM were working in parallel on GaAs/AlGaAs processing approaches. As result of this joint effort, a first successful etching protocol for V-groove etches has been realized serving as fiber alignment and mounting grooves (Figure 13a). A similar level of development has been achieved for Si-based dielectric chip-scale beam combiners. The structure of the combiners is based on OAP mirrors and is intended to guide the laser light through the silicon chip as shown in the COMSOL simulations shown in Figure 13b. This technology still requires optimization and will be implemented in the 2nd generation of MIRACLE devices.

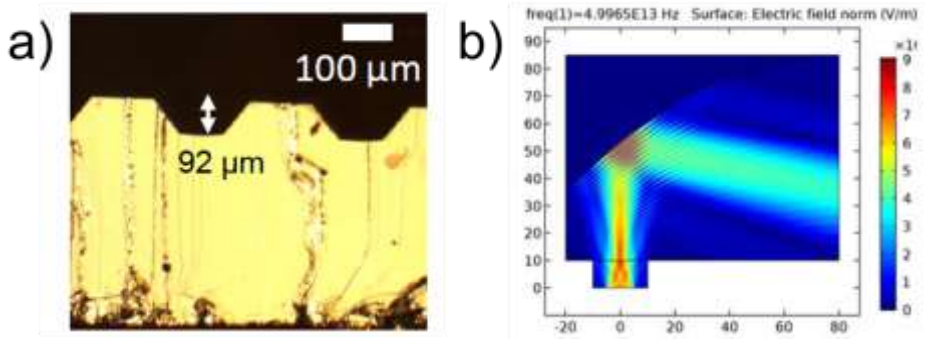


Figure 13 (a) V-groove trances for self-alignment of AgX fiber for on chip coupling (b) COMSOL simulation results for routing light within a dielectric chip-scale beam combiner.

Probe waveguide technology

Two options are possible to use – Polycrystalline MIR fiber with a good transmission in the spectral range 4-17 µm and Hollow Waveguides specially designed for the spectral range of interest. To compare the coupling properties of both waveguides a simple real probe has been built using HWG with inner diameter 1000 µm and its optical properties were compared with a similar standard probe made of PIR 900/1000 fiber core/clad diameter. The throughput of HWG based probe is as small as 8-10% of the standard probe measured with FTIR spectrometer Matrix MF (Figure 14).

The result is explained by low NA of all HWGs which cuts the radiation of high-order modes at the coupling with the FTIR spectrometer. Taking into account that the coupling mirrors in MIRACLE device provide a high NA as well, HWG does not match the goal of the project to build the probe with a high throughput.

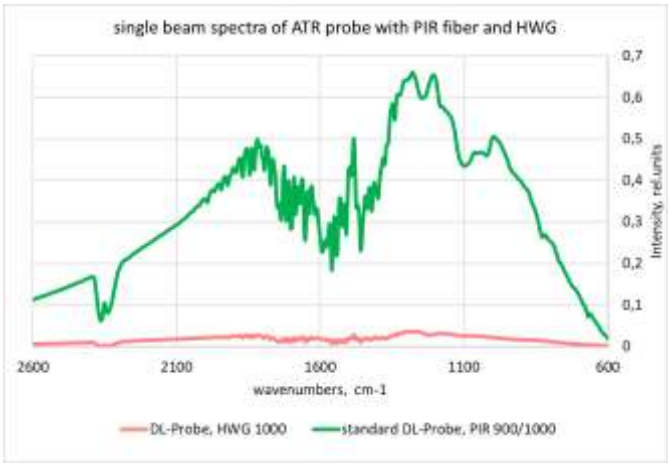


Figure 15 Comparison of the throughput of HWG based and PIR fiber based ATR probes.

Another measurement has been done with a single QCL and PIR 600/700 and PIR 900/1000 fibers to check the transmission of both options. Comparison of bending losses for PIR 900/1000 and PIR 600/700 fiber cable showed that thinner fiber provides higher throughput at the same bending radius (Figure 15).

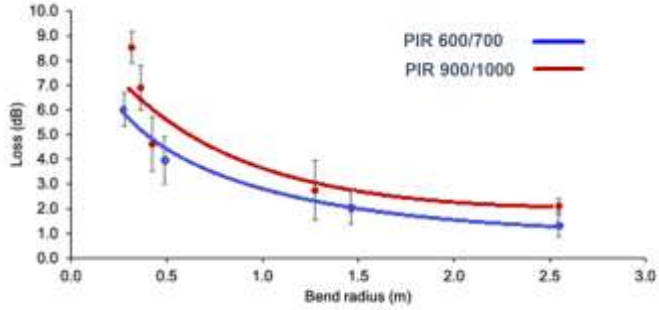


Figure 14 Comparison of the optical losses at the fiber bending with small radius: PIR 600/700 ------- and PIR 900/1000 ---

The coupling of the QCL in both PIR 600/700 fibers and PIR 900/1000 has been evaluated (Figure 16). The maximum transmission has been achieved for both fiber diameters. That means any of QCL can be effectively coupled into more flexible fiber with smaller diameter.

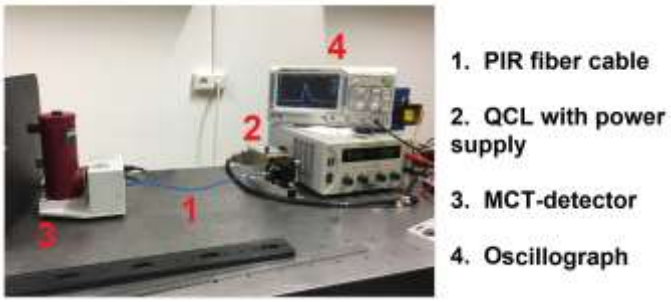


Figure 16 Evaluation of the fiber transmission with QCL

Design of an arthroscope probe based on the evaluation results.

This task started by focusing on the optical simulations regarding the geometry of the diamond ATR probe tip and the design of the fiber bundle. As mentioned earlier, PIR 600/700 was recommended for the assembly of the probe. To match the project requirements, the ray-tracing for the design with the mirror was simulated (Figure 17) and drawings were developed.

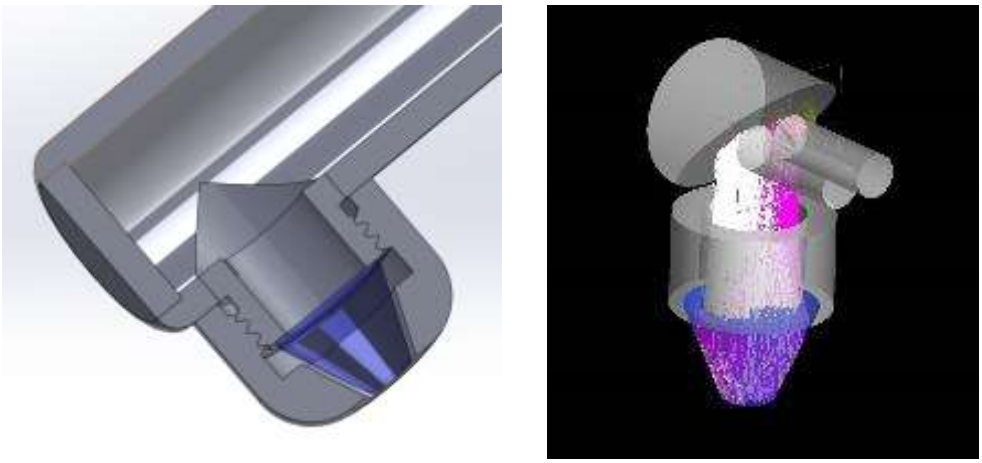


Figure 17a) Assembly drawing *b) specifications of the arthroscopic probe*

4.4. Main Unit

With respect to the main unit, several technical challenges were overcome. For the first time, 7 MIR QCLs were combined into one device. Their optical beams are coupled out through a single output port and coupled back in through an input port onto one MCT detector. In a first approach the coupling of the lasers was done outside the device. However, this turned out to be impractical. In a version 2 of the demonstrator, this coupling was integrated into the device. Figure 18 shows the device and the foot switch associated with initiating the measurement. (The surgeon should have a steady hand during the

Figure 18 Main unit with foot switch

measurement with the probe pressed onto the cartilage of the investigated joint and consequently not have to make a finger movement to press a button).

The lasers (from cooperation partner Nanoplus) are combined via a worldwide novel fiber optic coupling system (from cooperation partner art photonics and Ulm University) and focused into the removable, mechanically flexible infrared fiber cable with the attached handle part/probe (also from art photonics) on its distal end as depicted in Figure 19. The detection of the light coming back from the probe is realized via a commercial MCT detector. The major challenge of this arrangement was the combined electronic control of the lasers and the demodulation of the detector signal. On the one hand, a good signal quality (signal-to-noise ratio) was important, but the measurement speed should also be as high as possible, so that a surgeon can quickly scan the various areas to be examined during arthroscopy of a joint and obtain a reliable measurement value correlated to the degeneration of the tissue as quickly as possible. Finally, this electronic laser control and detector signal demodulation could be realized in-house by OptoPrecision. Via a software interface, which was also newly developed, this raw measurement data is transmitted continuously to the front-end software of the cooperation partner IRIS, which also runs on the device. In that software process, NMBU's AI-based data model is also integrated via a corresponding library. Furthermore, the human-machine-Interface is part of the IRIS software. Thanks to the excellent cooperation of all partners, a fully working integrated MIR-ATR arthroscopy device could be built, which is able to generate high quality measurement data.

Figure 19 shows the main instrument with a connected fiber cable and probe, as it is currently in use in the laboratory of the cooperation partner Ulm University. To avoid unwanted signal fluctuations, the flexible cable of the probe is held in place. In laboratory experiments, the tip of the probe is pressed in a defined manner onto appropriately prepared joint samples and the measurements are taken.

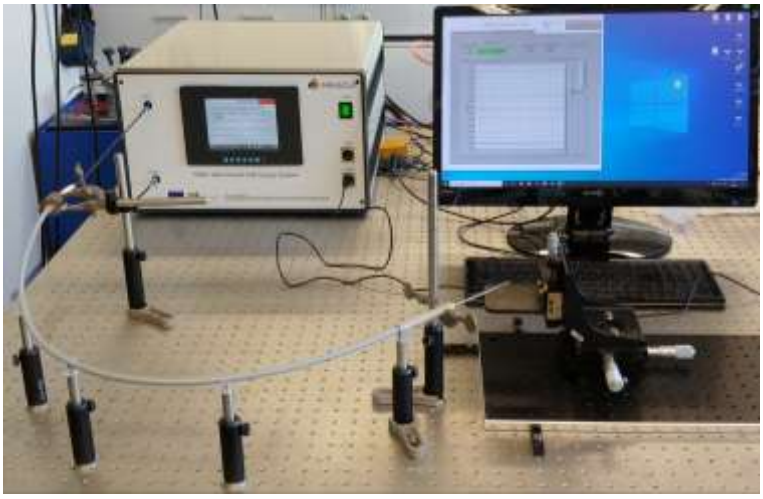


Figure 19 Main unit with attached fiber cable and handle / probe

4.5. Probe

Prototype development

The flexible fiber optic probe is a key component of the MIRACLE device to be used in an arthroscope and measure the cartilage damages. The probe needs to be shaped like a hook with defined dimensions (Figure 20). Additionally, the probe needs to be sterilizable by steaming and built of biocompatible/approved materials. The probe throughput is to be enough to provide a sufficient signal-to noise ratio at all key wavelengths chosen for the cartilage measurements.

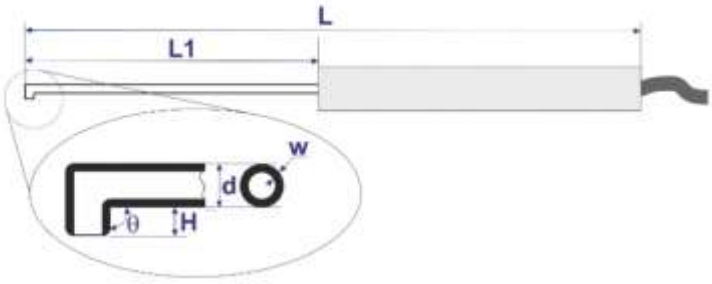


Figure 20 Probe requirements

Table 2 probe properties and requirements

Property	Requirements
Overal length L	Overall length (L): 210 mm (opt.: from 190 to 240 cm)
Shaft length L1	100 mm (opt.: from 90 to 110 mm)
Shaft tubing diameter d	3,5 mm (opt.: from 3 to 4 mm)
Shaft tubing wall w	0,75 mm (opt.: from 0,5 to 0,75 mm)
Hook height H	2 mm (opt.: from 1 to 4 mm)
Hook curvature θ	90 degrees

During the course of the project, a total of 11 probes were created. Each new prototype solved a different issue or accommodated an additional requirement. In total, 6 versions of the diamond probe were assembled, while more than 50 assembly attempts were made. Four probes were selected and shipped to project partners for the evaluation. In addition to the diamond probe, a fiber loop probe design was proposed and developed. Five versions of loop probes were manufactured and tested, including sterilization runs. But finally, a diamond probe design was chosen as it meets all project requirements and provides a stable signal, unlike the loop probe.

Overview of the prototypes

The biggest technical challenge was to build a mechanically strong probe with a hook. Table 3 summarizes the various prototypes' properties.

Table 3 probes' prototypes comparison

parameters	V.0 Straight Probe	V.1 Hook Probe	V.2 Hook Probe	V.3 Hook Probe	V.4 Hook Probe
Material	Titanium	Stainless steel	Hastelloy C22	Stainless steel	Stainless steel
Overall length	1.3 m	1.5 m	1.5 m	1.5 m	1.5 m
Shape	Straight	Hook shaped	Hook shaped	Hook shaped	Hook shaped
Shaft length	130 mm	100 mm	100 mm	110 mm	110 mm
Shaft diameter	3 mm	4 mm	4 mm	5 mm	5 mm
Tip diameter	3 mm	4 mm	4 mm	4 mm	4 mm
Hook height	N/A	2 mm	2 mm	3 mm	6 mm
Hook curvature	N/A	90 degrees	90 degrees	90 and 120	90 degrees
Hook fixation	N/A	Welding	Welding	Bending	Bending
Tip	CVD	CVD	CVD	CVD	CVD
Weight	200 g	250 g	250 g	200 g	200 g
Optical Fiber	2 x PIR Ag-X	2 x PIR Ag-X	2 x PIR Ag-X	2 x PIR Ag-X	2 x PIR Ag-X
Optical connectors	SMA	SMA	SMA	SMA	SMA
Disposable or reusable	reusable	reusable	reusable	reusable	reusable
Sterilization method	Autoclave	Autoclave	Autoclave	Autoclave	Autoclave
					

The V.0 Straight miniaturized Diamond ATR Probe was manufactured to provide the possibility for project partners to start measurements of cartilage samples as early as possible. A special flat tip Diamond crystal

has been designed and its sensitivity was tested and approved during the tests of this probe. The model of the 0th generation diamond probe is presented in Figure 21.

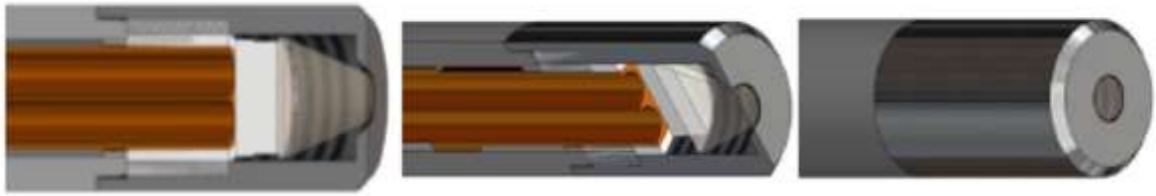


Figure 21 Internal design of the miniaturized Diamond ATR probe

The V.1 Hook Diamond ATR Probe was manufactured to modify the probe dimensions to project requirements. Fibers of the biggest standard diameter was used to achieve a satisfactory throughput. Unfortunately, the welding of the stainless steel shaft and tip was not strong enough to withstand the mechanical stress. Also, the handle made of PEEK polymer did not meet requirements. The 1st generation diamond probe suffered mechanical failure exactly in the weld seam in 18 months after the manufacturing at the attempt to try it in clinical settings. The model of the 1st generation hook Diamond probe is presented in Figure 22.

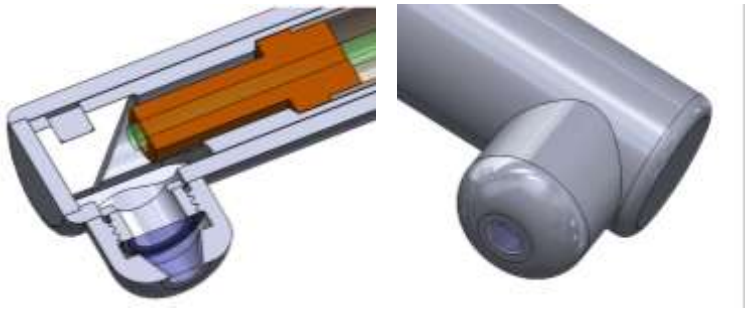


Figure 22 Internal design and appearance of the 1st generation hook-shaped arthroscopic probe

The V.2 Hook Diamond ATR Probe was manufactured using all materials in a full compliance with project requirements. All dimensions have been modified to be in line with the surgeons' needs. Fibers of the thinner diameter should achieve better flexibility. Hastelloy C22 material is approved for medical applications. Probe handle is made of stainless steel. The probe passed sterilization tests and showed a stable signal while used with MIR spectrometer. But the probe throughput is not very high, unfortunately, because of too complicated optical design. The model of the 2nd generation Diamond probe is presented in Figure 23.

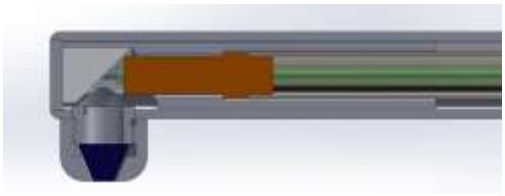


Figure 24 Internal design of the 2nd generation hook-shaped arthroscopic probe

The V.3 Hook Diamond ATR Probe manufacturing was performed adapting the probe design to solve the problem of mechanical strength and increasing the throughput. Based on multiple attempts to make a loop fiber probe the idea came to build the probe with bent fibers to deliver the radiation directly to the Diamond crystal. Moreover, a bent metal shaft would be much stronger mechanically in comparison with a welded one. Special tests have been performed to choose proper sealing materials for the probe in order to avoid infiltrations causing the fluctuation of the probe signal. After multiple attempts the probe was built but the throughput was low, unfortunately, even at 120 degrees bending. The efforts made at this stage helped to understand the fiber behaviour at the small radius bending. It helped to achieve better throughput in the V.4 fiber probe. Model of the 3rd generation Diamond probe is presented in Figure 24.

Figure 23 Schematic of the 3rd generation hook-shaped arthroscopic probe

The V.4 Hook Diamond ATR Probe. The improved hook diamond probe design was developed rethinking the hook shape to get a better signal. To complete the final diamond probe design, more than 10 attempts of assembling and performance test were done, which finally allowed to achieve an acceptable throughput. The signal of V.4 of the hook diamond probe is, in general, equal to the signal of a prototype straight Diamond probe. The 4th generation diamond probe was delivered to OP as the final version for integration with the main unit. The model of the 4th generation diamond probe is presented in Figure 25.

Figure 25 Schematic of the 4th generation hook-shaped arthroscopic probe

Probe evaluation

The 4th generation diamond probe's performance was evaluated following the standard certification procedure used in art photonics for product quality control. The Total Transmission value allows to compare the probes and provide data for the preliminary assessment of the transmission at MIRACLE device. The comparison between different generations of diamond probes is shown in Figure 26.

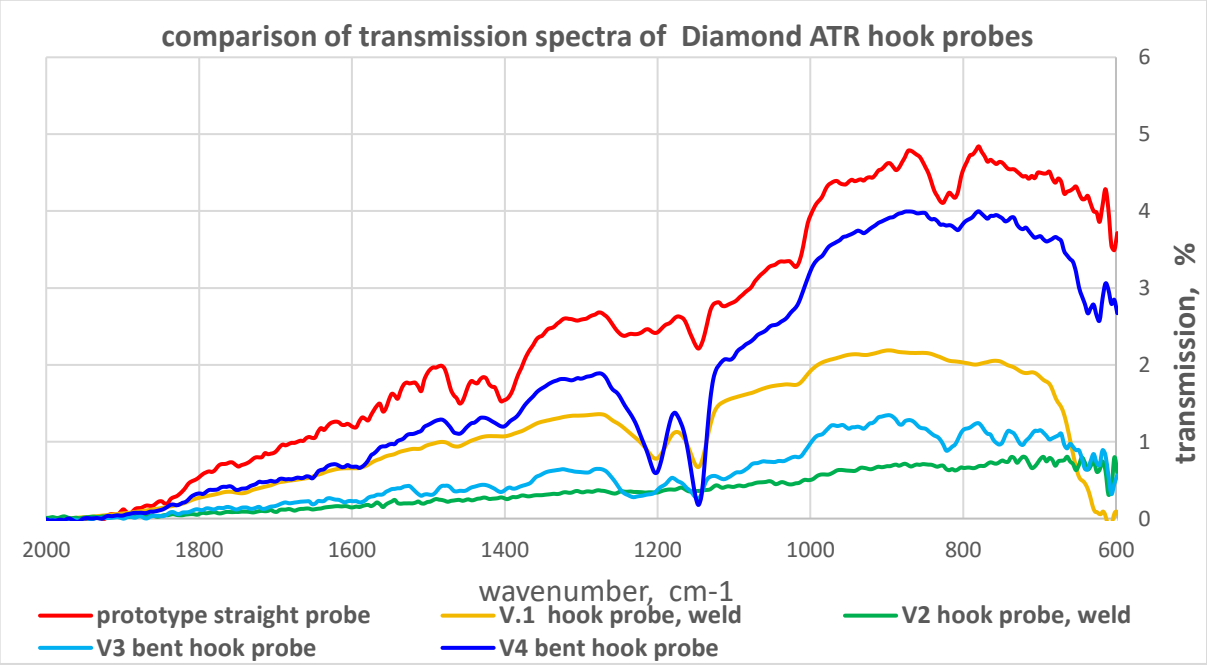


Figure 26 Comparison of transmission of the 1-4 generations of hook-shaped diamond probes .

The low signals observed for 2nd and 3rd generations diamond probe are explained by small fiber diameter in comparison with the 1st generation, low coupling efficiency fibers-to-diamond in 2nd generation probe and small fiber bending radius in 3rd generation probe.

The 4th generation diamond probe has a design with a bent shaft. It means the fibers are to be bent with a small radius to direct the radiation into the Diamond crystal and therefore to the sample. The main differences in the 4th generation probe are as follows:

- The shaft material is stainless steel as required
- Outer shaft diameter is 4 mm
- The probe hook is about 1.5 mm longer as required to keep the fibers is a right position inside the probe and maintain a satisfactory signal level.

The probe hook is about 1.5 mm longer as required to keep the fibers is a right position inside the probe and maintain a satisfactory signal level. Overall, the 4th generation bent probe design is more suitable for medical application due to the robustness compared to the Ist and 2nd weld versions. The test with MIRACLE device showed the QCL beams transmission are not significant lower than for the straight MIRACLE prototype Diamond Probe.

4.6. Data Analysis and Modelling

Modelling of real life data is always a challenge. There are many different aspects of the data processing which should be addressed. Analysis of sparse data, such as the data of MIRACLE device comprising only seven wavenumbers, brings new challenges to this process.

One of the challenges in dealing with sparse data is pre-processing. Pre-processing involves removing unwanted variation in the data that is due to white noise and other instrumental effects. The pre-processing of broadband spectral data is known to improve data modelling results, at least when classical machine learning methods are used. Up to now, very limited attention was given to preprocessing of sparse IR spectral data. Therefore, our task was to optimize the preprocessing of the sparse MIRACLE data.

The quality of the obtained spectra is another issue in spectroscopy, which is barely addressed in any scientific discussions. This is a very important step in the analysis as data recorded by the instrument may not contain relevant spectral signatures, for example due to poor contact of the probe with the cartilage. When the contact of the probe is bad, a low-quality spectrum containing mostly water or synovial fluid surrounding the cartilage, is recorded. Therefore, preselection of cartilage-specific spectra is of extreme importance for the diagnosis of the cartilage quality during the medical operation.

In addition to preprocessing and preselection of the spectra, modelling of data with only few wavenumbers is also challenging. The majority of multivariate analysis methods are optimized to work with highly multidimensional data comprising hundreds or thousands of spectral variables. Therefore, methods that work successfully with only sparse data had to be selected and modelling approaches had to be optimized.

Variable selection

Seven wavenumbers for the production of custom-made fixed-wavelength QCL lasers for the MIRACLE device had to be selected. The choice of the wavenumbers was based on the results of multivariate modelling techniques, such as sparse partial least squares discriminant analysis (SPLS-DA) and random forests (RF) models, the relevance of the wavebands for the discrimination of the cartilage quality, as well as the usefulness of the wavenumbers for the preprocessing of the spectra prior to the modelling. The following wavenumbers were thus selected: 1800, 1745, 1620, 1560, 1210, 1080, and 850 cm^{-1}. The positions of the preselected wavenumbers are shown on the broadband spectrum of human cartilage in Figure 27.

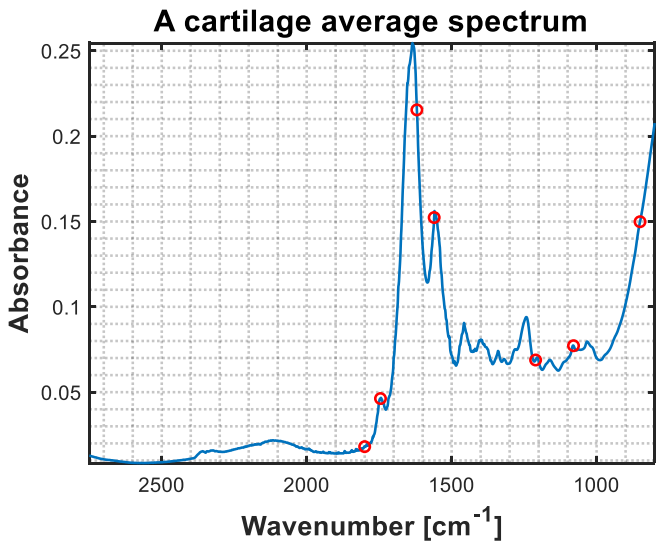


Figure 27 An average broadband spectrum of human cartilage obtained using human data in blue and the seven selected wavenumbers shown by red circles. The wavenumbers 1800, 1745, 1620, 1560, 1210, 1080, and 850 cm^{-1} were selected based on their relevance to cartilage quality assessment and spectral pre-processing.

Five variables, 1080, 1210, 1560, 1620, 1745 cm^{-1}, are cartilage-specific:

- 1745 cm^{-1} corresponds to the C=O stretching vibration of lipids present in the cartilage and synovial fluid,
- 1620 cm^{-1} corresponds to the C=O stretching vibration (amide I) of collagen.
- 1560 cm^{-1} corresponds to the C-N-H stretching and bending vibration (amide II) of collagen.
- 1210 cm^{-1} corresponds to the O=C-N-H stretching and bending vibration (amide III) of collagen,
- 1080 cm^{-1} corresponds to the C-O stretching vibration of carbohydrate residues in collagen and proteoglycans.

Two variables, 1800 cm^{-1} and 850 cm^{-1}, were selected due to their value for pre-processing of the spectral data.

During the project period, we tested the discrimination power of the selected variables and observed that they are highly relevant for the discrimination of the cartilage quality and support pre-processing of the

sparse data. The results are summarized in the manuscript[1] where models using human, bovine and simulated sparse data were used to classify samples to healthy and damaged cartilage groups (healthy OARSI <2, damaged OARSI >=2). Overall, good models classifying healthy from damaged cartilage samples were obtained. When optimal pre-processing was applied to the sparse spectra with only seven variables, models' performance did not drop significantly compared to the models based on the broadband spectra for the data available to the project. These observations, however, were based only on the sparse data (seven wavenumbers) of the broadband ATR-FTIR spectra (i.e., the reference infrared measuring device), since the necessary MIRACLE device-based sparse data (QCL data) were not yet available.

Pre-processing

In preparation of the preprocessing of the QCL data, data for the respective seven wavelengths were selected from ATR-FTIR spectra and pre-processing tools were developed with this data. The two wavenumbers selected for the pre-processing purposes, namely 1800 cm^{-1} and 850 cm^{-1} were proven to be relevant for their purpose.

The first wavenumber, 1800 cm^{-1}, was selected as a reference variable for the baseline absorbance, since the spectral region between 2750 and 1780 cm^{-1} is mostly devoid of strong chemical absorbance by the samples. Also, strong absorbance caused by the ATR diamond crystal between 2500 and 1900 cm^{-1} results in a relatively low signal-to-noise ratio in this region.

The second variable at 850 cm^{-1} is a band related to water in the cartilage and synovial fluid. This wavenumber was selected as a reference variable since it is assumed that the chemical absorbance of water is mainly invariant in all the samples, and therefore it could be used for normalization of all measured variables.

The pre-processing of the sparse spectra of the broadband ATR-FTIR data was optimized using human and bovine data. The optimization of the pre-processing was based on the classification performance of the PLS-DA models discriminating healthy and damaged cartilage (healthy OARSI <2, damaged OARSI >=2). As the result, the optimal pre-processing was baseline correction by 1800 cm^{-1} and normalization by 850 cm^{-1}. In some cases, it was observed that the commonly used model-based preprocessing method multiplicative signal correction (MSC) can also be used to pre-process the spectra.

Due to delays linked among other to the COVID pandemic and the restrictions it brought, pre-processing of the QCL data and the OARSI grading are still ongoing at the time of the writing of this report.

Spectral pre-classification

Another activity related to the quality of data and spectral pre-processing is so-called spectral pre-classification. During the project, NMBU has conducted a study where we developed a technique to preselect the high-quality spectra. A high-quality spectrum is defined as a spectrum containing strong

[1] Tafintseva, V., et al., *Preprocessing Strategies for Sparse Infrared Spectroscopy: A Case Study on Cartilage Diagnostics.* Molecules, 2022. **27**(3): p. 873.

(noticeable) cartilage signals. We have observed that some spectra recorded in the project were clearly spectra of water solutions surrounding the sample. Such spectra do not contain any relevant information regarding the cartilage and can therefore be ignored. The water spectra can for example be obtained due to poor contact of the sample and the measurement probe. With the pre-classification algorithm in place, the operator - doctor - receives a warning and is advised to repeat the measurement by either changing the position or obtaining better contact with the cartilage. While infrared broadband spectra of cartilage and water can usually be visually distinguished from each other, sparse data containing only seven wavenumbers does not allow the same visible control. In any case, the MIRACLE system needs an automatically performed pre-classification of collected spectra, to be able to remove spectra that do not have any diagnostic value. The technique developed by NMBU works for both full-range spectra and sparse data. The developed technique is based on MSC and has proven to be a robust method for spectral pre-classification. The results are summarized in a manuscript which is ready for submission[2].

Classification models

Using ATR-FTIR broadband spectra and sparse spectra (seven wavenumbers of the broadband spectra) of different dataset available in the project we have proved that cartilage damage diagnostics based on infrared device is possible[3]. It still remains to check whether real QCL data, obtained by the MIRACLE device, will result with good discrimination diagnostics. We have strong reasons to believe so, since MIRACLE QCL data and ATR-FTIR spectra of the same samples showed good correlation (meaning good agreement in the spectral features detected by different spectroscopic techniques).

When the reference OARSI data and QCL data are finally available (expected in the last weeks of the project), the models will be built for QCL data. The partners are currently working on the data acquisition of both QCL and OARSI reference data.

[2] Rehman, H.U., et al., *Pre-classification of broadband and sparse infrared data by multiplicative signal correction approach.* Molecules, New Winds in Chemometrics: Theory and Application, submitted.
[3] Virtanen, V., et al., *Infrared Fiber-Optic Spectroscopy Detects Bovine Articular Cartilage Degeneration.* Cartilage, 2021. **13** (2_suppl): p. 285S-294S.

4.7. Global Control System and User Interface

An interactive and user-friendly interface

An interactive and user-friendly interface has been developed for the MIRACLE device utilization; including the corresponding architectures, features and decision-support components for both the cloud and the desk formats. The global control system will host all the data acquired from the devices connected. Also, the collected data is synchronized with the Hospitals IT infrastructures through the cloud services, although the user can also work offline by the use of an USB stick. These mentioned architectures have been acquired based on experts' medical opinions regarding different testes configurations and mock-ups. Figure 28 shows and schematic view of the general architecture.

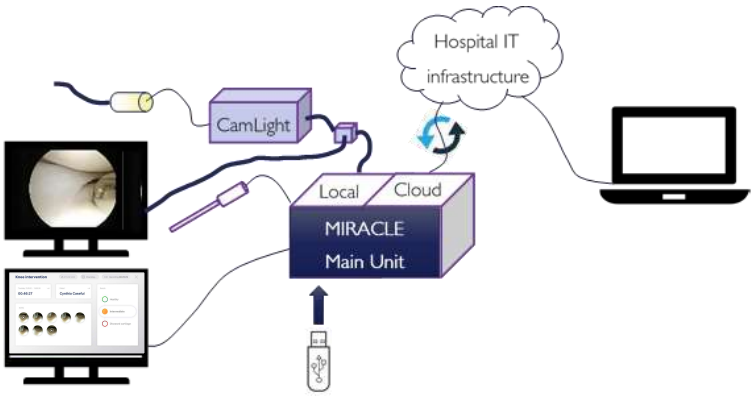


Figure 28 Schematic representation of the general software architecture.

The software was developed based on a hybrid-architecture approach in which a local-cloud server runs alongside with a desktop software. This hybrid-architecture allows to combine the reliability of the local instance with the accessibility that a SaaS method offers. Also, the MIRACLE software has been optimized seeking a teamwork-friendly, which increases the effectiveness and efficiency when upgrades are introduced. It will include a fast video processing feature as well as an intuitive user interface. In addition. the hybrid-architecture brings different operating modes depending if the user is operating online or offline, understanding online as a remote connection to the software; and offline as the functionalities being available and accessible only by the users interacting with the local device.

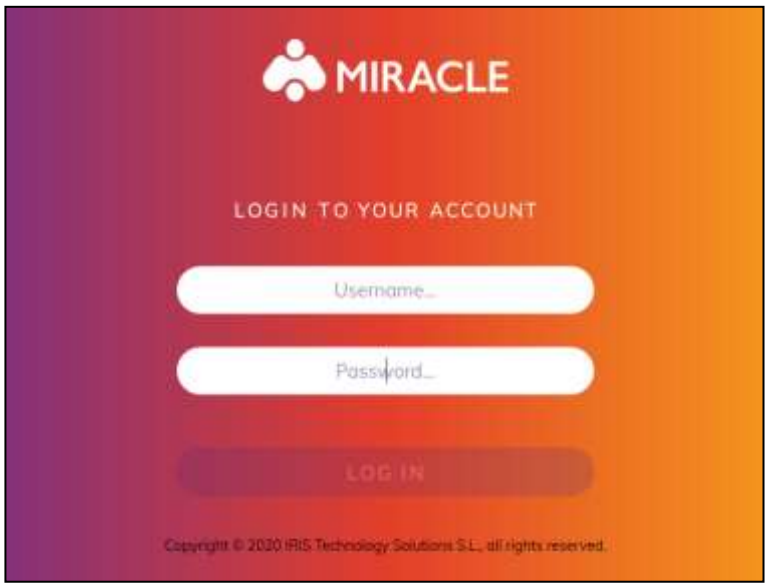


Figure 29 Initial screen of the MIRACLE software.

After introducing the corresponding credentials in the interface shown in Figure 29, different operating modes can be accessed depending on the user (patient, medical staff and maintenance staff) and the operating purposes. The operating modes can be summarized as follows:

- Online mode: the standard operational mode, which is managed by the patient for historical results consultation. It works on the local-cloud environment and is independent from the local device.
- Offline mode: it allows the operation of the device when the connectivity is not possible. This mode will be extendedly used by the medical staff since its reliability on private-networks connectivity is considerably low. This operative mode will be also used in emergency situations and when needed in-field measurements must be taken in absence of connectivity. In the case of medical staff. Figure 30 shows the interface view from the medical staff when consulting historical results filtering by patients or surgery.
- Surgery mode: it is an automatic mode that takes action when the surgeon starts a surgery. Whitin this mode, the GUI is fully dedicated to providing *in-situ* information, i.e., view from the arthroscopic camera and video stream. Also, the interaction between the software and the

incorporated models will provide a traffic-light based response with the suggested health state of the analyzed joint. Figure 31 shows a few features seen when a knee sample is analyzed.

- Maintenance mode: specifically designed to be operative when software and models updating and maintenance actions are being performed. It is mainly used for accessing to detailed information regarding the system.

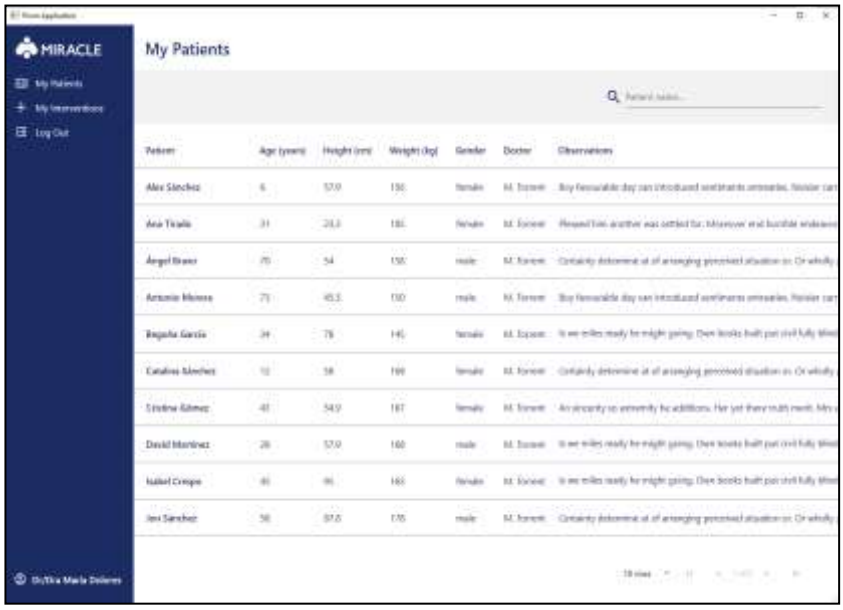


Figure 30 Medical staff view when accessing the MIRACLE software for historical results filtered by patient.

Figure 31 Left: traffic light system showing the result after the analysis has been performed. Right: Screenshot arthroscope guided probing of damaged articular cartilage

The interaction between the MIRACLE device and the developed software was carried out in continuous communication with the partners OP (In charge of the LabView hardware controller) and NMBU (in charge of the multivariate modelling), in association with the previously mentioned experts' opinions.

In order to achieve an optimal functionality from the software functionalities to the medical staff needs, the data provided by the hardware level are offloaded to the models while the software uses the exact same data to trigger the procedures to be stored and to finally show the results to the screen.

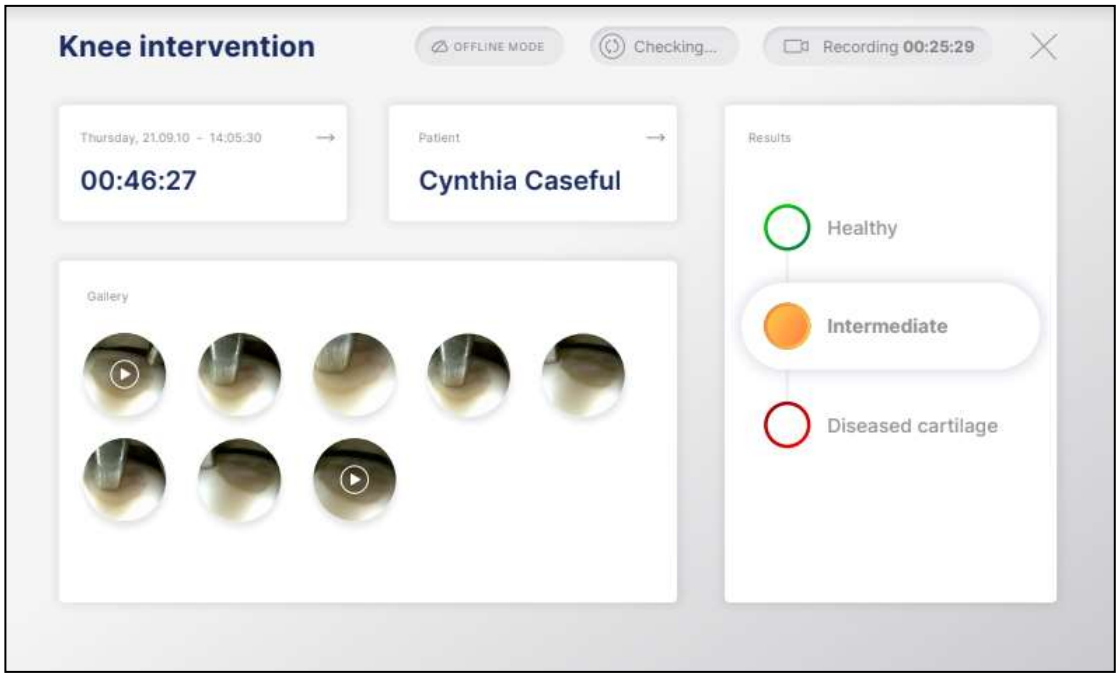


Figure 32 Screenshot of the tested interface when the surgeons can revise the data achieved, including the videos captured, the operation and recording time, the patient details and the corresponding response from the models applied to the measurement.

The resulting measurement of the models processing is embedded with the video stream provided from the arthroscopic camera; and it is stored in a single video file together with the record that the surgeon saw during the evaluation. Figure 32 shows the GUI design along with the corresponding features and videos stored. Finally, the data is identified with a unique ID code, and it is shared with the local-cloud that will make them reachable through a unique URL.

4.8. Demonstration and Validation

The work was carried out as a joint effort from several partners: UOULU, UEF, NMBU, UU, KUH and UULM. 1864 sets of human, bovine, equine, and sheep cartilage tissue samples were successfully collected and measured with four different MIR spectroscopy devices. During the project, other connective tissues such as human meniscus and ligaments were also successfully tested with MIR spectroscopy. MIR-ATR measurements were conducted to determine the ability of the methodology to detect different levels of tissue damage and disease progression. OARSI grading was used to determine the level of tissue damage in the samples. Other reference measurements, i.e. polarized light microscopy (PLM), digital densitometry (DD) and biomechanical measurements were also performed for some of the samples.

Pre-validation via *in vitro* studies

Bovine tissue study

Bovine articular cartilage was used as an experimental model of mechanically and enzymatically degenerated tissue to mimic PTOA and OA respectively. Cylindrical osteochondral samples from 6 different anatomical location of 12 patella were divided into control, enzymatically degraded and mechanically degraded groups. Samples were analyzed using Art Photonics MIR-ATR probe with Thermo Nocolet iS50 FTIR spectrometer. To investigate the capability to discriminate between different experimental degradation types from MIR spectra, Random Forest, PLS-DA and SVM-DA models were trained. The results from these experiments demonstrate the ability of MIR-ATR method to detect tissue-level damage to articular cartilage with very good accuracy.

Equine tissue study

Equine samples for study were harvested from 6 slaughter horses (Van de Veen, Nijkerk, The Netherlands). Fetlock joints of both forelimbs and both hindlimbs were isolated and samples were taken from the third metacarpal/metatarsal bone (MC3/MT3 and the proximal phalanx (PI)). Cartilage from all the knees were visually assessed according to the ICRS grading system prior to cutting the samples for FTIR measurements. The FTIR analysis was done at UULM with Bruker ALPHA I FTIR ATR spectrometer. The reference measurements were done at UEF and after histologically processed at OULU. SVM-DA model was trained and validated with prediction set. Classification was based on ICRS gradings of the sample. All 4 grades (0-3) were predicted individually, and the performance of each grades' prediction were pooled into one. Overall performance of MIR-ATR classification was similar to the bovine dataset.

Sheep meniscectomy study

Sheep meniscus samples were acquired from left knee of five sheep after six-month post meniscectomy. Meniscectomy was performed on the lateral side of the medical meniscus in the parts intermediary region. As healthy reference, the right knee was kept intact. ATR spectra of the meniscus sample were acquired with a Bruker ALPHA II FTIR ATR spectrometer at UULM. The MIR spectra of the partial meniscectomy meniscus sample shown a significant peak decrease in the region of amide I band. Furthermore, the amide II bands show as a red shift of the meniscectomy samples as well as an increase and decrease at peak maximum. C-H bending bands show a decrease of the shoulder band of the meniscectomy samples. These results indicate a decrease of the triple helical structure of the collagen content of the meniscectomy samples.

Human tissue

Cartilage study

Human tissue was utilized to validate the method for differentiating osteoarthritic tissue from healthy tissue. In the main study, an extensive set of samples from tibial, femoral and patellar osteochondral tissue was analyzed.

Three sets (76 cylindrical samples (d = 8 mm) from 2 cadavers, 281 cylindrical samples (d = 4 mm) from 9 cadavers and 180 cylindrical samples (d = 4 mm) from 9 cadavers plus 80 cylindrical samples (d = 4 mm) from 8 biobank knee joints) of articular cartilage were prepared with a dental drill from central location of femoral, tibial and patellar cartilage.

The first two sets of samples were measured with MIR-ATR at UULM. Spectra were recorded with a Bruker ALPHA I FTIR ATR spectrometer. These two sample sets utilized for evaluation of MIR-ATR methodology were processed for histological grading in UOULU after measurements in UULM. These histological sections were evaluated according to the OARSI grading system, which grades the lesion depth to assess the severity of OA with grades ranging from 0 (healthy) to 6 (severe OA with bone exposed).

A partial least squares discriminant analysis (PLS-DA) classifier was used for discriminating the spectra into healthy (OARSI 0-2) and osteoarthritic (OARSI 2.5-6) groups. A good overall classification accuracy of

74.6 % was obtained with a PLS-DA model. This is a promising result considering the limited amount of data for modelling in the current study and the conservative method applied to validate the developed PLS-DA model. The present results indicate that an objective spectroscopic approach could provide an improvement over the currently used subjective methods, which have a limited accuracy.

The third set of samples (180 cylindrical samples (d = 4 mm) from 9 cadavers plus 80 cylindrical samples (d = 4 mm) from 8 biobank knee joints) were raw spectra measured with the MIRACLE device (7 wavelengths) and with control measurements using Bruker ALPHA II FTIR ATR spectrometer (full MIR spectra). Similarly to the two first sets of samples, this set was provided to histology grading to OULU. All data collected in UULM and OULU were utilized in development of the PLS-DA model by NMBU which will be used in MIRACLE device.

Meniscus study

Samples of lateral meniscus specimen were taken from 24 patients at the University and Rehabilitation Hospital in Ulm. The dataset comprised 16 women and 8 men, with an age median of 67.1 ± 9.0 years. The samples were graded with the Kellgren and Lawrence score. The cylindrical meniscus samples had a diameter of about 4.6 mm. The meniscus samples were collected at the inner meniscal circumference from various sections of the human meniscus specimen. Prior to FTIR studies, the samples were lyophilized and stored at room temperature. Additionally, the samples were smoothed at both sides using sandpaper (1200/2400), to achieve a smooth contact area for the ATR crystal surface. ATR spectra of the meniscus samples were acquired with a Bruker ALPHA I FTIR spectrometer.

The amide I band of the evaluated samples shows a significant blue shift of the peak maximum from 1635 cm^{-1} (grade 1) to 1639 cm^{-1} (grade 4 with calcification). Especially, the peak intensity at about 1660 cm^{-1} increases with increasing degeneration of samples. The results show that the degeneration process affects a wide range of involved biomolecules. I.e., the random structure of the involved proteins remains mostly unaffected by the degeneration processes. On the contrary, triple helixes and alpha-helixes appear to be significantly affected by degeneration processes of the meniscus. The analyzed spectra indicate an extension of the triple-helical structure. In summary, a decrease of water binding proteoglycans and an increased density of the collagen fibril network can be concluded.

Ligament study

Samples of ACL, LCL, PCL and MCL ligaments were taken from 8 biobank cadaver knee joints. Spectra of 3 points of each ligaments were recorded using a Thermo Nocolet iS50 FTIR spectrometer equipped with single ATR crystal. These ligaments sample sets utilized for evaluation of MIR-ATR methodology were processed for histological grading in UOULU after measurements in UEF.

4.9. Communication and Promotion

Communication toolkit and website

A main objective of the Consortium was to keep the communication channels up-to-date, promote and give visibility to the MIRACLE project. During the project timeline, the communication strategy was continuously worked to make the project's communication appealing and effective.

Under INEB's coordination and with the collaboration of all consortium partners, a website was designed to ensure its communication goals while delivering a user-friendly experience (https://miracleproject.eu/). A final version was launched on 30 June 2020 (Figure 33). The architecture was structured to organize and deliver the content to different target audiences: general public/patients, surgeons/doctors and business/companies. The design of the website was set to be optimized and responsive for multiple devices (computer, tablet and smartphone). The results, articles and activities of MIRACLE was made available in the News section of the website. The website had a total of 7,364 visitors with 21,540 views and has been updated with 105 project-related news.

Figure 33 MIRACLE project website

A Communication Toolkit was prepared at the beginning of the project, which includes the general guidelines (e.g., visual guidelines, logos and fonts), documents templates (e.g., Word documents, PowerPoint slideshows and posters) and material for dissemination and communication (e.g., Roll-up banner, exhibition stand and flyers).

Three different flyers were designed: for patients, surgeons and companies. The flyers designed for patients were translated into French, Spanish, Russian, Portuguese, Norwegian, and Dutch. The toolkit was continuously updated through the project

Figure 34 Communication toolkit materials

Social media

In order to increase the online presence, MIRACLE launched the official Twitter (twitter.com/H2020MIRACLE) and Facebook (www.facebook.com/H2020MIRACLE/) account to reach the stakeholder through other channels. The content from the website, including news and project updates were shared on social media. Since the launching of the social networks in July 2020, 134 posts were published, gathering together 538 followers, 74,003 impressions and 3,283 engagements. Articles and

content from other related social network pages (e.g., Optics & Photonics Days 2021) relevant to the MIRACLE project's audience were shared through the social networks. A series of videos, entitled "The faces behind the MIRACLE (project)", was prepared in which each partner presented themselves and their role in the project. Both the project-related news and videos have been shared on the partners' websites and social media channels, including Facebook, LinkedIn, Twitter, and YouTube. An intranet, based on Basecamp, was used as means of internal communication and for sharing data and other relevant materials within the consortium.

Conferences and Symposia

As another way to further promote MIRACLE's results, the consortium partners participated and delivered presentations at different scientific conferences (see summary in Table 4).

Table 4 Summary of scientific conferences and summer schools, as well as in outreach events

Event		Type of Communication	Participants
01	7th Workshop on Nanocarbon Photonics and Optoelectronics (NPO2018)	Scientific workshop	105
02	Advanced Photonics 2018 in Clinical Medicine and Surgery	Scientific event	n/a
03	European Researchers' Night at University of Oulu	Fair/Exhibition	1000
04	EU Workshop on Performance Assessment and Standardization in Biophotonics	Workshop with other EU projects organized by EC	n/a
05	Biophotonics for Healthcare Event (2019)	Workshop organized by MIRACLE dedicated to MIRACLE dissemination	71
06	9th Nordic Cartilage Imaging Meeting	Scientific Conference (Oral presentation)	40
07	ANAKON 2019	Scientific Conference	560
08	OSA Biophotonics Congress: Optics in the Life Sciences	Scientific Conference	n/a
09	Imaging optical spectroscopy seminar	Scientific Conference	10
10	SPIE Photonics West	Scientific Conference	23 000
11	BiospecMLC 2019: Machine Learning and Chemometrics in Biospectroscopy	Workshop	50
12	Workshop Performance Assessment and Standardization in Biophotonics	Workshop	40
13	OARSI 2019 World Congress	Scientific Conference (Oral presentation)	2000
14	16th Science Day of the Campus of Kontinkangas (2019)	Scientific Conference (Poster Presentation)	n/a
15	Optics & Photonics Days 2019	Scientific Conference (Poster roll-up)	242

16	Laser World of Photonics 2019	Trade Fair (Poster roll-up)	33999
17	BioSpecMLC 2019: Machine Learning and Chemometrics in Biospectroscopy, Minsk, Belarus	Worshop (Oral presentation)	50
18	XXII. Annual Linz Winter Workshop 2020	Scientific Conference	150
19	SPIE Photonics West BIOS 2020	Scientific Conference	23,000
20	Finnish Physics Days 2020	Scientific Conference	10
21	11th World Biomaterials Congress (WBC2020)	Scientific Conference	1,100
22	29th Annual Meeting of the European Orthopaedic Research Society (EORS)	Scientific Conference (MIRACLE Symposium; Exhibition Stand)	400 (250 Online)
23	The XXV Saratov Fall Meeting 2021	Scientific Conference (Oral presentation)	130 (70 Online)
24	Optics & Photonics Days 2021	Scientific Conference (Oral presentation; Industrial session presentation; Exhibition Stand)	150

The MIRACLE consortium organized the "BioPhotonics for Healthcare Workshop" that took place on 13th and 14th of March 2019 at the Tellus Stage, University of Oulu, Finland. The workshop provided high quality education for the next generation of photonics scientists and promoted an active collaboration amongst H2020 funded projects, researchers, clinicians and companies. The event was attended by speakers from Finland, Germany, Italy, France, Norway, Lithuania and UK.

Figure 35 BioPhotonics for Healthcare Workshop promotion flyer

A closing conference at the end of the project was forecasted to promote the MIRACLE device and ensure the project outcomes achieve the highest impact. However, due to the COVID-19 pandemic, it was decided to join an existing event.

The 29th Annual Congress of the European Orthopaedic Research Society (EORS 2021- Rome, September 15-17) was the selected event. The MIRACLE consortium organized, at this event, the symposium "A Bright New Future for Arthroscopy".

The symposium was chaired by Prof. Simo Saarakkala (University of OULU) and Prof. Gabriela S. Lorite (University of OULU), and the panel was composed of Prof. Boris Mizaikoff (University of Ulm), Prof. Harold Brommer (University of Utrecht), and Prof. Ali Mobasheri (President of the Osteoarthritis Research Society International (OARSI).

Figure 36 MIRACLE Symposium and Exhibition Stand at EORS2021

Scientific articles

During the project, 18 scientific articles were published and 2 thesis were defended (Table 5). With the support of the consortium members - surgeons, physicists, and engineers - new findings were made, enhancing the project outcomes.

Table 5 List of publications

#	Title	Journal	Citation	Partner
01	Analytical Performance of μ-Groove Silicon Attenuated Total Reflection Waveguides	Analyst	DOI: 10.1039/C9AN00417C	UULM
02	Analysis of human menisci degeneration via infrared attenuated total reflection spectroscopy	Analyst	DOI: 10.1039/C8AN00924D	UULM
03	Surface analysis of sheep menisci after meniscectomy via infrared attenuated total reflection spectroscopy	Journal of Biophotonics	DOI: 10.1002/jbio.201800429	UULM
04	Mid-infrared GaAs/AlGaAs micro-ring resonators characterized via thermal tuning	RSC Advances	DOI: 10.1039/C8RA10395J	UULM
05	iBEAM: substrate-integrated hollow waveguides for efficient laser beam combining	Optics Express	DOI: 10.1364/OE.27.023059	UULM, NP, OP, AP
06	Nanotechnological Strategies for Osteoarthritis Diagnosis, Monitoring, Clinical Management, and Regenerative Medicine: Recent Advances and Future Opportunities	Current Rheumatology Reports	DOI: 10.1007/s11926-020-0884-z	UOULU
07	Machine learning augmented near-infrared spectroscopy: In vivo follow-up of cartilage defects	Osteoarthritis and Cartilage	DOI: 10.1016/j.joca.2020.12.007	UEF, UH, UU
08	Infrared Fiber Optic Spectroscopy Detects Bovine Articular Cartilage Degeneration	Cartilage	DOI: 10.1177/1947603521993221	UOULU, UEF, KUH, NMBU
09	Raman spectroscopy is sensitive to biochemical changes related to various cartilage injuries	Journal of Raman Spectroscopy	DOI: 10.1002/jrs.6062	UEF, KUH, UOULU
10	Near Infrared Spectroscopy Enables Differentiation of Mechanically and Enzymatically Induced Cartilage Injuries	Annals of Biomedical Engineering	DOI: 10.1007/s10439-020-02506-z	UEF, KUH, UOULU
11	Preprocessing Strategies for Sparse Infrared Spectroscopy: A Case Study on Cartilage Diagnostics	Molecules	DOI: 10.3390/molecules27030873	NMBU, UULM, UOULU, UEF

12	Infrared spectroscopy is suitable for objective assessment of articular cartilage health	Osteoarthritis and Cartilage Open	DOI: 10.1016/j.ocarto.2022.100250	UOULU, UEF, KUH NMBU, UULM,
13	Preclassification of Broadband and Sparse Infrared Data by Multiplicative Signal Correction Approach	Molecules	DOI: 10.3390/molecules27072298	NMBU, UOULU, UEF, KUH, UULM
14	Structural, compositional, and functional effects of blunt and sharp cartilage damage on the joint: A 9-month equine groove model study	Journal of Orhopedic research	DOI: 10.1002/jor.24971	UEF, UU
15	Tissue optical properties combined with machine learning enables estimation of articular cartilage composition and functional integrity	Biomedical Optics Express	DOI: 10.1364/boe.402929	UEF
16	Quantitative dual contrast photon-counting computed tomography for assessment of articular cartilage health	Scientific Reports	DOI: 10.1038/s41598-021-84800-x	UEF
17	Characterization of connective tissues using near-infrared spectroscopy and imaging.	Nature Protocols	DOI: 10.1038/s41596-020-00468-z	UEF
18	Articular cartilage optical properties in the near-infrared (NIR) spectral range vary with depth and tissue integrity	Biomedical Optics Express	DOI: 10.1364/boe.430053	UEF
19	Preprocessing strategies for infrared spectral data with limited numbers of spectral channels	Master Thesis	-	NMBU
20	Advanced waveguide technologies for enhancing the performance of infrared chemo/bio sensors	PhD thesis	DOI: 10.18725/oparu-31169	UULM

Communication achievements

The major Communication achievements are summarized in Table 6

Table 6 MIRACLE communication achievements

Achievements since the start of the Project
The MIRACLE website had a total of 21,540 views and 7,364 visitors.

A total of 105 news were published on the website, with in addition two press releases (one concerning the project kick-off at M2 and one about the synergy between RESTORE and MIRACLE projects at M18).
134 post were published in the social platforms (Facebook and Twitter), gathering a total of 538 followers, 75,003 impressions and 3,283 engagements. In addition to MIRACLE social channels, partners used their social media network (i.e., INEB-i3S' Facebook) to share and expand information and news related to the project.
The MIRACLE project organized the Biophotonics for Healthcare Workshop event, and the Symposium "A Bright new Future for Arthroscopy" (held at the EORS 2021). Since the start of the project partners attended to 24 events (e.g., conferences, workshops, etc.)
The MIRACLE device was promoted in two events, at the Optics & Photonics Days 2021 and at the EORS 2021.
A series of videos, entitled "The faces behind the MIRACLE (project)", was prepared in which each partner presented themselves and their role in the project.
Consortium members published 18 scientific articles and defended 2 theses.

4.10. Business and Exploitation Plan

Stakeholder Engagement, liaison with other EU funded projects

Engagement of stakeholders is essential to obtain feedback to design the device to the needs and requirements of the end-users and to the specifications and views of the buyers. The main stakeholders of MIRACLE include orthopaedic surgeons, hospitals, specific companies, insurance companies, as well as veterinary surgeons and researchers with interest in the topic, specifically those working in the areas of tissue regeneration. Ultimately, patients who may benefit from arthroscopy performed using the device are also stakeholders who should be informed on the capabilities of th device.

The MIRACLE website pages dedicated to surgeons and companies were developed to contain selected information directed at its stakeholders. The presence on social networks also aims to raise awareness of the project and create a network to facilitate communication with stakeholders. Moreover, the MIRACLE device was presented in an exhibition stand at the 29th Annual Congress of the European Orthopaedic Research Society (EORS 2021), a symposium organized by the project partners, "A Bright New Future for Arthroscopy", was held at the same event. The MIRACLE device was also presented at the Optics & Photonics Days 2021 on an exhibition stand and during an Industrial session presentation. Several stakeholders were engaged in these events, in particular surgeons and enterprises working in the area of medical devices for orthopaedic surgery.

Also, questionnaires were sent to orthopaedic surgeons to get feedbacks on their views on the MIRACLE arthroscopy device being developed. The interviews with surgeons performed including over 95 contacts reached through digital platforms, direct interviews and congress participation, about 15 interviews were performed with orthopaedic surgeons from different regions in Europe.

Intellectual Property management

The MIRACLE project offers opportunities for new innovations. Those are partly generated within the consortium as a joint effort, and partly as individual partner´s in-house process. Innovation activity is one of the key indicators for the success of the project.

IPRs have an essential role in the entire life cycle of the MIRACLE project. Proper management of IPR enables an effective exploitation of the research results.

The key innovation management activities are:

- monitoring of innovation actions,
- supporting partners in enhancing their innovation capacity, and
- integration of new knowledge.

During project implementation, the evolution of the background and foreground knowledge was monitored annually. Two partners brought to MIRACLE project four patent families as their background.

In addition, partners were asked to inform the current IPR situation of potential new IPR. To this date, MIRACLE project has not produced any new registered IPR. More developmental and testing work is still needed in order to get solutions to the point where patents can be applied. However, MIRACLE project has produced software that are automatically protected by copyright.

Annually patent database searches were done during the project. The aim was to know

- the competitive advantages of solutions developed in the MIRACLE project
- how to tune solutions developed in the MIRACLE project in order to get clearer competitive advantages?
- Can the results of MIRACLE be patented?
- Is there Freedom-to-Operate (FTO) i.e., bring the developed solution(s) to the market without need to get a license to third party´s patent right?

Based on deep analysis of publications found from patent database searches, none of them was found to create problems for the consortium in terms of the novelty of the solutions developed in MIRACLE or for their commercial exploitation..

Exploitation and Sustainability

The MIRACLE project involves 4 companies partners focused on the development of components of the medical device for use in joint arthroscopy, namely AP, NP, OP and IRIS. Individual Business Plans were prepared for each partner, enabling to refine the position of each partner, to estimate costs and revenues, as well as the preliminary commercialization strategy.

To ensure a sustainable and profitable use of the project's results after project end, the MIRACLE partners developed an Exploitation Plan for all Key Exploitable Results that can be exploited during and after the project. This includes a preliminary analysis of the Key Exploitable Results in terms of uniqueness, needs

and requirements for market entry and market analysis. This Plan was upgraded as the project evolved and the necessary technical information on the exploitation results became available, and the market analysis was expanded to include a deeper analysis of the markets for human and veterinarian uses, and the business models for their exploitation. A market overview and technological trends was performed to get a clear picture of the current market and to assess the strengths and weaknesses of the MIRACLE project as well as all the market opportunities and the risks associated with the development of a new technology associated with a medical device.

In the arthroscopy market, it was estimated that almost 2 million people in the US and 6.1 million in the EU underwent an emergency arthroscopy due to sport-related reason. The most common arthroscopy in humans are associated with knee injuries. On the other hand, the veterinary market has strong possibilities associated with equine interventions. A list of Key Opinion Leaders, key commercialization partners and key players was developed targeting the aforementioned markets.

Pathway for regulatory approvals

The Consortium reviewed the regulatory approach for the MIRACLE category of devices and solutions in the marketplace and impact on end-user, policy, business models and dependent factors for commercialization, providing the partners with information about the relevant documentation requirements for the regulatory approval of the different MIRACLE products.

The first step in determining the most appropriate regulatory pathway to obtain clearance or approval of a new medical device in both the EU and US markets is to develop a robust regulatory strategy by aligning the business objectives and the regulatory requirements necessary for the market launch of the product.

During the project, the MIRACLE products classification was defined and the strategy for regulatory approvals was built in line with the exploitation plan led by UCP. All the required standards were provided by UNE, and the steps and documentation necessary to submit to regulatory bodies for the different MIRACLE devices were detailed in MIRACLE's Pathway for Regulatory Approval. In brief, MIRACLE outcomes include two different sets of products: (a) Qualified and marketed as medical devices (i.e. there is an intended medical purpose): MIRACLE device (i.e. the integrated unit, the probe and the software. (b) Qualified and marketed as components for medical devices: QCLs and the beam combiner solutions. To obtain CE Marking certification, the MIRACLE device must comply with European Commission Regulation (EU) No. 2017/745, commonly known as the Medical Device Regulation (MDR). According to the MDR, all the MIRACLE medical devices (as described in (a)) are classified as Class IIa. The general safety and performance requirements, the technical documentation, the conformity assessment, and the clinical evaluation were determined in accordance with the proposed classification. Gaining access to the EU market first presents the advantage of the technical documentation required for regulatory approval in the EU market largely fulfils other markets' regulatory requirements.

Standardization

Standardization activities in MIRACLE are considered as a way to increase the impact of the project in European industry, Public Administrations and Society. The activities performed are aimed to:

- Contribute to the generation of new standards covering outcomes of the project.
- Use the standardization system as a focused dissemination channel.

MIRACLE has proposed and developed a CEN/CENELEC Workshop Agreement CWA 17857:2022, "Lens-based adaptor system for coupling fiber optic to infrared semiconductor lasers" (www.cencenelec.eu/media/CEN-CENELEC/CWAs/RI/cwa17857_2022.pdf)

The key steps in the development of the CEN-CENELEC Workshop Agreement are summarized below:

- Proposal preparation: The proposal was sent to four Technical Committees from CEN, CENELEC, ISO and IEC (CEN/TC 123 - Lasers and photonics, CLC/TC 76 - Optical radiation safety and laser equipment, ISO/TC 172 - Optics and photonics, IEC/TC 76 - Optical radiation safety and laser equipment), which did not submit any objection to the creation of the Workshop. Finally, it was announced in the CEN-CENELEC webpage in 2021-08-03 for a 30 day public commenting period, which did not register any comment.
- Workshop Kick-off meeting: Held in 2021-09-08 with participants from in and out the project consortium.
- Drafting stage: Different rounds of commenting and redrafting were linked to obtain by December 2021 an agreed draft.
- Public Commenting Stage: A 30 day period of public commenting was launched by CEN and CENELEC. Some comments were received from some ISO/TC 172 members (from Japan and USA). Comments were considered, some of them accepted, leading to small changes in the final draft.
- CWA Publication: After approval, the definitive CWA draft was sent to CEN-CENELEC, with the final publication in February 2022.

The CWA will be available for a maximum of 6 years. After this maximum period, it could be updated into a EN or ISO standard or take part of a new standard covering several methods for coupling fiber optic to lasers (as suggested in one of the comments received from ISO/TC 172).

Publication by the European Standards Organizations, CEN and CENELEC, of a standardization document such as a CWA is a successful outcome for a Horizon 2020 project. Visibility has been given to MIRACLE and a new standard has been made available, which can be used by industry players in different sectors and applications of semiconductor lasers, for instance, medical equipment, environment, pharmaceutic, biotechnology, process optimization and many more. This way, part of the knowledge generated by the project has been transferred to industry and can revert into technical and economic benefits for Europe.

Additionally to this standardization activity, communication has been held with the standardization Technical Committees on "Electrical equipment in medical practice", CLC/TC 62 at European level and IEC/TC 62 at international level. These committees are composed by all the stakeholders interested in

electromedical devices participating through the equivalent national committees in the 34 European countries in the case of the CENELEC/TC and in 49 international countries (27 of them non-European) in the case of the IEC/TC. This wide group includes manufacturers but also users, services providers, public administrations, testing laboratories, researchers, technology developers, etc. Only in Spain, the national mirror committee counts with more than 20 participating organizations. Therefore, this communication about MIRACLE reached largely 1000 different worldwide related organizations.

An e-mail was sent on 2021-11-24 to the Secretariats of both Technical Committees, including a presentation about the project facts, objectives and technological developments. Secretaries circulated the information to the participants. A supporting response was received from the Chair of IEC/TC 62/SC 62D 'Electromedical equipment' in the following terms: ''After reviewing different articles, yes, indeed, this is a project which can be done in IEC/TC 62/ SC 62D.''. This is of course good news for the MIRACLE Team, that can in the future, once the device is fully operative and the required regulatory pathway fulfilled, they have the option of pioneering the standardization of the new technology, with the competitive advantadges this implies.

5. Conclusion

The MIRACLE project was a very ambitious research and development project in its technological approach and the formulated implementation towards a medical device. All project participants, external technical experts, project reviewers as well as the EU project supervisors were aware of the enormous scientific challenge from the very beginning.

The project has yielded a considerable amount of scientific knowledge, which is also reflected in numerous publications. The business success, which is a key element for the companies involved, is for its part unfortunately not (yet) as tangible as originally hoped. There are however commercially viable results that can be further exploited and developed into new applications.

In science, theory and applicability do not always go hand in hand, and although the project made major breakthroughs, a number of impasses and technical difficulties arose during the course of the project, hindering the fully completion of the final device. To solve these, the partners involved have been constantly adapting their work packages and workflow, going to the extent of self-financing additional expenses when they felt this could lead to a viable solution. Regrettably, at the end of the project, we are not yet there. As a result, we have to admit that we won't be able to achieve all the goals set 4 years ago at the beginning of the EU funded MIRACLE project.

What does this mean for everyone involved?

- The goals realized (lasers, multi-laser main unit with signal detection) lead directly to economically usable partial products, but not jet to a medical device, which is able to assign a cartilage structure the proper health status / OA grading value.
- Assuming that the ongoing measurement data analysis is successful and a mathematical model for reliable correlation between measurement data and OA grading can be created, the partners will attempt to achieve the originally set goals via a follow-up project.
- If the data evaluation is not satisfying or the required precision and robustness cannot be achieved for the originally planned medical purpose, there are still other promising fields of application for the realized measurement technology in the area of scientific research and also in the industrial environment, for example in quality monitoring in the food and beverage industry. Initial discussions have already been initiated regarding a self-financed continuation of the development work in these directions.

Ultimately, the technological risk in this project, which also justifies the public co-financing, could not be completely controlled even with the greatest efforts of all partners within the given budget and duration. But even though the final device might not be achieved, new technologies with a wide range of potential applications were created and scientific limits were pushed.

6. Annexes

6.1. Annex 1: MIRACLE folder for patients

MIRACLE

Detecting early cartilage damage.

Osteoarthritis: A serious disease

Osteoarthritis is a joint disease resulting from the breakdown of joint cartilage and underlying bone. It contributes to the global disability and affects an estimated 242 million people worldwide. A large part of this population is senior which is highly susceptible to bone diseases will substantially drive the demand for these devices.[1]

MIRACLE device solution

MIRACLE is the first mid-infrared attenuated total reflection (MIR-ATR) arthroscopy imaging system for real-time, in-depth, clinical examination and diagnosis of degenerative joint diseases, that can be used in clinical cases where patients are recommended to undergo arthroscopy surgery.

Promoting patient well-being

MIRACLE device will allow orthopaedic surgeons to obtain real-time information about the biochemical composition of the cartilage tissue, leading to objective decision-making on the most adequate treatment course, enhancing patient's well-being and reducing the need for follow-up surgery.

Facts on Osteoarthritis

242 million people affected globally

50% over 65 affected worlwide

8.9 years estimated delay for diagnosis

up to **€10,452** per year / patient in EU

1 – March et al. Osteoarthritis: A Serious Disease. Submitted to the U.S. Food and Drug Administration. 2014.

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 780598. The project is an initiative of the Photonics Public Private Partnership.

Annex 1I:

Empowering surgery practice to promote patient well-being

The concept behind MIRACLE is quite simple. Cartilage tissue is made of very specific molecules. Some molecules have particular response when illuminated with a specific light source (as for example specific lasers). This phenomenon is called molecular vibration and it can generate a frequency spectrum, which works as a molecular 'finger print' for the cartilage tissue.

Healthy and damaged cartilage have different spectra. Also, different damage levels show different spectra. Based on these scientific evidences, MIRACLE device will be capable of compiling this information into a colour-coded map of healthy and damaged tissue during the arthroscopy procedure.

The surgeon will then have all detailed information in a graphical user interface to decide whether further action has to be taken. The device is not capable of doing external examination and will be used only during arthroscopy.

How does it work

The device will measure the biocomposition of cartilage tissue. This can be achieved by using biophotonics, i.e. the combination of biology and photonics. Photonics is an area of study that involves the use of radiant energy (such as light, lasers and so on) whose fundamental element is the photon.

QCL | IBEAM | Main Unit | Probe | User Interface | Diagnosis

Consortium

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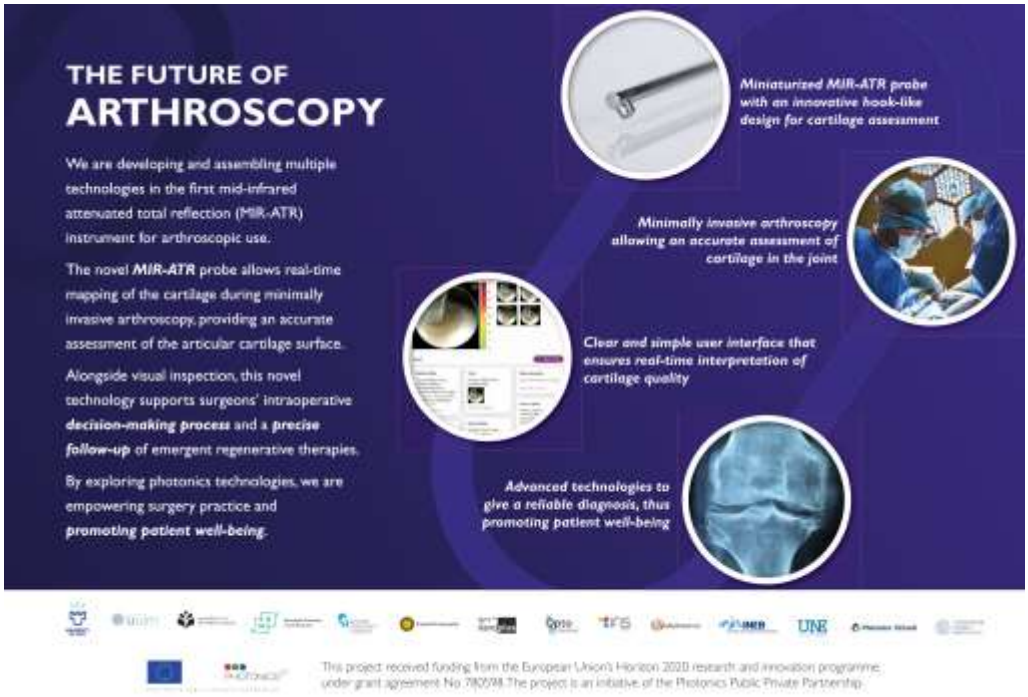
6.2. MIRACLE folder for surgeons



6.3. MIRACLE folder for companies