EDOCAL

DEMONSTRATOR

Early Detection Of CAncer using Lasers

Final Publishable Summary Report

November 2015

The research leading to these results has received funding from the European Union Seventh Framework Program (*FP7/2007-2013*) under grant agreement no. 605254

EXECUTIVE SUMMARY

Cancer, found in the mouth, lips or throat, is often highly curable if diagnosed and treated early. Unfortunately, in its early stages, oral cancer can easily go unnoticed as it is often not visible using conventional imaging techniques. In fact even when a lesion is visible to the naked eye, there is no way for the dentist to see the difference between a cancerous and benign lesion.

The core objective of the EDOCALD project is to deliver an optical imaging device which creates an image of the oral cavity (broad field white light detection) and maps within the oral cavity highly suspicious lesions with performance targets of >95% sensitivity and specificity using our narrow field tunable laser detector. The output of our Demonstrator provides not only the image of the suspicious lesions but also the option to scale recorded images such that the progession of lesions over time can be monitored. Most oral lesions are benign, but many have an appearance that may be confused with a malignant lesion, and some previously considered benign are now classified as premalignant because they have been statistically correlated with subsequent cancerous changes. Conversely, some malignant lesions seen in an early stage may be mistaken for a benign change. Our system is intended to be able to differentiate cancerous lesions from a multitude of other red, white, or ulcerated lesions that also occurs in the oral cavity.

Results from the EDOCAL Research Project showed that it is possible to discriminate pre-cancerous tumours even before they are clinically visible mucosal changes, allowing a more confident assessment of risk and localization of the most suspicious area to biopsy. Absence of auto-fluorescence was found to be sufficient to identify mucosal dysplasia however some areas of non-fluorescent lesions can still give false positives (leading to poorer specificity). This led us to conclude that we would need to combine our EDOCAL detection method with narrow and broad field detection modes and a statistical model which would be used to characterise the different data groups collected and correlate these with known outcomes.

The purpose of the EDOCAL-DEMONSTRATOR was to build the first generation Demonstrator of a real-time Photonic Cancer Detector (PCD) that will initially be used for early diagnosis of oral cancers. Any oral lesion that does not regress spontaneously or respond to the usual therapeutic measures should be considered potentially malignant until histologically shown to be benign. A period of 2 to 3 weeks is considered an appropriate period of time to evaluate the response of a lesion to treatment. When our system takes a second image of the patient it can overlay the images and see if lesions from first image are still present or if new lesions have appeared or if the size has changed. When lesions are present in both images and are identified as pre-malignant or malignant then a definitive diagnosis is warranted (biopsy). This will reduce the number of unnecessary biopsies taken and hence reduce costs.

The current system has detected malignancy with 100% sensitivity and 100% specificity. With further data collection and analysis we are targeting being able to apply the same technology to discriminate pre-malignant tissue that will become malignant and pre-malignant tissue that will not become malignant while it is still in the pre-malignant state.

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1 Project Context and Objectives

1.1 Context

Early detection of cancer saves lives, improves quality of life and reduces health care cost.

In EDOCAL, a Research for the Benefit of SMEs project (Contract No. 231993) that was concluded in December 2011, Proof of Concept was achieved for early detection of cancer based on the use of tunable blue semiconductor laser technology. The technology has been tested and proven for oesophageal cancer detection and can be used for early detection of many other types of cancer including mouth, stomach and colorectal.

In cancer, tissue cells tend to create many additional blood vessels to support their growth. The molecule protoporphyrin (PpIX) is generally present in blood vessels and exhibits red fluorescence when excited with UV or blue light in the 360-425 nm range. Cancer cells and pre-cancer cells are detected by observing the red fluorescence at a matching excitation wavelength**. The wavelength of the laser has to be tunable as the matching wavelength depends on the person, type of illness and presence of other chemicals.** Baselines are created using surrounding healthy tissue.

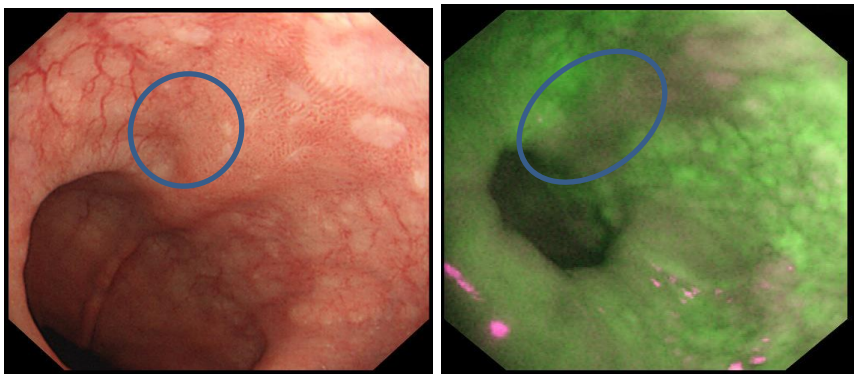

Figure: 1.1: Malignant tissue encircled and surrounded by healthy tissue under standard white light endoscopy (left), fluorescence endoscopy (right).

During the period 01.01.2010 to 31.12.2011, the SMEs 2M Engineering Ltd, Compound Semiconductor Technologies Global Ltd and TopGaN Ltd worked together with 2 RTDs: Ninewells Hospital in Dundee and Amsterdam Medical Centre on the EDOCAL project. Results from the EDOCAL prototype showed that it is possible to discriminate pre-cancerous tumours even before they are clinically visible mucosal changes, allowing a more confident assessment of risk and localization of the most suspicious area to biopsy. Absence of auto-fluorescence was found to be sufficient to identify mucosal dysplasia however some areas of non-fluorescent lesions can still give false positives (leading to poorer specificity). This led us to conclude that we would need to combine our EDOCAL detection method with narrow and broad field detection modes and a statistical model which would be used to characterise the different data groups collected and correlate these with known outcomes.

The purpose of this project is to use the results of the EDOCAL project and extend the system to incorporate the narrow and broad field detection modes and a statistical model, building a demonstrator that will enable the first product commercialisation 9 months after completing this project.

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1.2 Project Objectives

In making a choice of the first application we addressed the issues that would be important success criteria in moving our technology from research to commercialization. We decided to focus our technical solutions on a stand-alone configuration which can be applied directly and easily by healthcare staff, similar to standard imaging systems today. In addressing these criteria we conclude that our first system would be best used in the screening of Oral cancers. It can be applied in the dentist offices as part of standard dental check-ups. People with a high risk of oral cancer are more likely to benefit from oral cancer screening.

Factors that can increase the risk of oral cancer include: Tobacco use of any kind, including cigarettes, cigars, pipes, chewing tobacco and snuff, heavy alcohol use; previous oral cancer diagnosis.

The high risk group is large and easily identifiable by the dentist. Worldwide over 640,000 new cases are diagnosed with oral or pharyngeal cancer each year. Oral cancer is annually responsible for more than 124,000 deaths. Of those 640,000 newly diagnosed individuals, only approximately 57% will be alive in 5 years. **This is a number that has not significantly improved in decades.** Historically the death rate associated with this cancer is particularly high not because it is hard to discover or diagnose, but due to the cancer being routinely discovered late in its development. Today, there is still no comprehensive program to opportunistically screen for the disease, and without that; late stage discovery and high mortality rates are most likely to continue.

Therefore the opportunity for EDOCAL-DEMO is to build the first generation Demonstrator of a real-time Photonic Cancer Detector (PCD) that will initially be used for early diagnosis of oral cancers. Cancer, found in the mouth, lips or throat, is often highly curable if diagnosed and treated early. Unfortunately, in its early stages, oral cancer can easily go unnoticed as it is often not visible using conventional imaging techniques. In fact even when a lesion is visible to the naked eye, there is no way for the dentist to see the difference between a cancerous and benign lesion. **Our core objective is to deliver an optical imaging device which creates an image of the oral cavity (broad field white light detection) and maps within the oral cavity highly suspicious lesions with performance targets of >95% sensitivity and specificity using our narrow field laser detector.** The output of our Demonstrator will provide not only the image of the suspicious lesions but also their spatial co-ordinates within the cavity (saved as a digital file). Optionally we may offer a means for the dentist to shine the map back into the cavity at any time in order to further evaluate progression after a few weeks or in order to take a biopsy.

The steps we needed to take to achieve our goal can be summarized as follows:

1. Use as input the data, intellectual property, application know-how, algorithms, lasers, detection and control systems and experience from the earlier EDOCAL Research project that was used to successfully arrive at a proof of concept for the early detection of cancerous tissue.
2. In the first six months of the project, FOCE and 2M designed and built the first generation Demonstrator using unique components from TG and CST. This was used to get functional performance feedback from the medical practitioners, selected clinicians (lead customers), (potential) marketing partners and a number of key industrialization partners. This feedback enabled us to complete the technical

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specifications for the final Demonstrator system. We used this feedback to partly re-design and build the 2nd generation Demonstrator that during the second half of the project. This was then used for clinical testing, early market development, determination of the production processes and the supply chain logistics for all components.

3. FOCE and UNIVDUN used the 1st generation Demonstrator to finalize the detection scheme and implement the algorithms necessary for the specific application and laser specifications desired.
4. The laser specifications were used by TG and CST to select the correct materials and processes to allow them to produce the laser devices to be used in the 2nd generation Demonstrators. Both TG and CST are the key suppliers of the GaN wafers and tunable laser module respectively for the final products.
5. UNIVDUN functionally tested the 1st generation Demonstrators and determine test protocols during the first half of the project. In the second half of the project UNIVDUN completed the first clinical tests using the 2nd generation Demonstrator. UNIVDUN has access to a large patient population and therefore in this project we planned to screen more than 100 patients and catalogue information on more than 1000 lesions. UNIVDUN also delivered indicative protocols for clinical studies to be started after the successful conclusion of this project.
6. 2M created the Demonstrator marketing/sales plan. The Demonstrator sales are planned to commence 9 months after successful completion of this project. Therefore during the project needed to work on infrastructure for marketing/sales, customer support, service, production, sourcing, medical conformity, certification, etc. Some of these activities were funded separately by the partners but are mentioned here to give a better overview.
7. In developing the application we create numerous inventions from which we have applied for one new patent and are planning more so that these patents, together with our basic patent will build significant barriers to potential competitors in addition to increasing competitive advantages.

1.3 Project Team

The core business of 2M is the generation of new product ideas bringing these ideas from concept to commercialisation. An example is a patented sensor system for measuring wear and tear on carrier bands in compressors for the gas and oil industry. Knowing exactly when the bands need to be replaced means that the maintenance can be optimised, replacing the bands too early is very expensive, a typical maintenance run costs about €2M. The system is ATEX Certified and is in production at 2M. 2M is project co-ordinator and responsible for building the demonstrators (software, firmware, electronics and optics), developing the marketing plan and taking the lead in the exploitation of the results.

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CSTG is a leading provider of III-V opto semiconductor solutions since 2000. CSTG will provide customised tunable lasers and arrays of blue lasers for use in the Demonstrators. CSTG provides laser knowledge and expertise in the area of laser tunability. CSTG will supply micro-fabrication expertise and capability for the development of GaN chip-level solutions based on the optimisation of a ridge waveguide Fabry Perot laser diode platform.

TopGaN was founded in 2001 by a private investor EuroCityII and the Institute of High Pressure Physics UNIPRESS belonging to the Polish Academy of Sciences. The goal of TopGaN is to develop and manufacture new generations of GaN-based laser diodes (LDs). TopGaN will supply the epi-material for blue laser arrays and will provide support for laser wavelength selection and providing test samples.

The Photobiology Unit (PBU) in Dundee is based within the Department of Dermatology, Ninewells Hospital and Medical school. The Unit has extensive experience of serving industry and has gained considerable expertise in the design, conduct and interpretation of photo(chemical)toxicity clinical studies. The Unit benefits from a multidisciplinary mix of clinicians, photo-physicists and photo-biologists approximately half of whom are directly employed in clinical research. In addition to work on human volunteers and patients, laboratory facilities allow a range of light-drug *in vitro* interaction studies to be conducted, producing high quality clinical and pre-clinical photo-toxicity data. The university of Dundee will be responsible for the pre-clinical test and validation of the demonstrators as well as the clinical testing and data collection.

FOCE International Technology BV

The core business of FOCE is consulting to electronics industry laser design in the US and in Europe. FOCE has provided designs for a wide range of electronics applications from semiconductor physics to system integration, software applications, integrated laser optical systems and mechanical design concepts. Over 50 patents have been generated. Specific experience is in the design of a novel blood centrifuge for the generation of platelet rich plasma in collaboration with 2M. The centrifuge included a novel chamber design that permits a low cost disposable and integrates modern sensor processing techniques. FOCE also has experience in laser design and bio-fluorescence analysis. Dr. Marcel Schemmann (FOCE) will also be responsible for the technical direction and the interface with suppliers.

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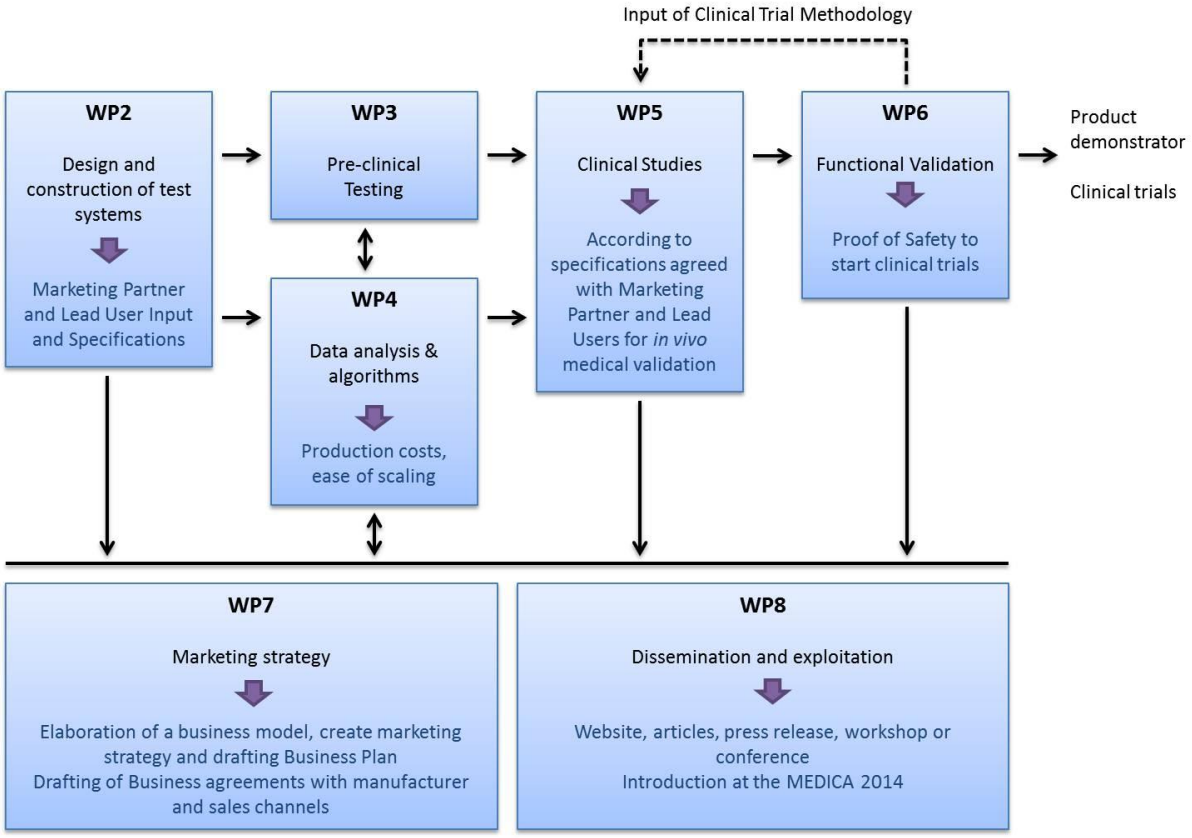
2 Main Scientific and Technological results

2.1 Project Overview



2.2 WP1 Project Management

2.2.1 Description of Work package 1

WP1 organizes the overall project, giving guidance to the other 7 work packages. 2M will take the lead to manage this project. Any non-technical issues such as interfacing with external parties, including the EU will be handled in this WP. WP1 is also intended to organize the IP related issues at the earliest possible opportunity so that they can be handled effectively from early on and throughout the entire project. All partners are expected to contribute to this work package. WP1 will have the responsibility of setting up a website which will be used to host project information both for the project partners as well as the general public. WP1 will also be concerned with writing, integrating and providing the interim and final project reports.

2.2.2 Work carried out in WP1

- The project website was set up in month 1 and is updated on a regular basis.

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- The consortium agreement was completed and signed by all of the partners in month 2.
- The background IP check was completed in month 2 and the updated report on IP was completed in month 10. We enlisted the services of an experienced patent attorney to support the investigations. A second patent has been filed for the probe we designed for the system and we continue to look at more opportunities for patent filing as we continue to analyse more patient data. We also continue to investigate possibilities for protecting the system more extensively through not only patent applications but also through barriers we can build into the supply chain, such as the use of key (exclusive) components and not publishing the algorithms used.
- Arranging for Ethical Clearance took longer than planned. Initially we planned to have this completed by month 3 but the clearance was only granted in month 7. The actual testing on patients could only start on April 29th due to delays with getting all of the components for the system and arranging indemnification for the University of Dundee to use the demonstrators.
- Project progress meetings progressed very well. The first meeting (project kick-off) was held in Dundee where the new project participants met for the first time. The second meeting was held at 2M in January 2014. This meeting was planned to coincide with the first Milestone (M1) completion of the Demonstrator Specification. This document was reviewed by all of the partners and since then has been under the supervision of the CCB (Change Control Board). The third Consortium meeting was planned so as to receive initial feedback from the Medics one month after the system was delivered. The system was delivered by 2M on April 29th and the third consortium meeting was held in Dundee on May 30th. The fourth meeting was held at 2M to coincide with the project review meeting. Further consortium meetings were held on: 23.02.2015, 30.06.2015 and 18.09.2015 alternating between Dundee and Valkenswaard.
- There are bi-weekly meetings between 2M and FOCE who have been working together intensively since the start of the project on designing, building and testing the first version of the demonstrator. There are also regular telcos between CST and TopGaN about the laser technology, packaging and performance. Ninewells Hospital and the Dundee Dental school are working closely and carrying out complementary tasks, Ninewells characterising the system in the laboratory and the Dundee Dental school using the system in the clinics, collecting the patient data. Both Ninewells and the Dental school provide feedback not only on the performance of the system but also on the usability on patients and in the clinic. The feedback from use in the laboratory setting has already been incorporated into the system and the feedback from use in the clinical setting will be incorporated into the final version of the demonstrator.
- All deliverables have been completed, not all of them on time during the second reporting period due to components being delivered too late, modules getting lost in transport and problems getting the demonstrator working in Dundee due to problems that could not be reproduced in The Netherlands.

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2.3 WP2 Design and construction of Test Systems

2.3.1 WP2 Supply of high performance Blue laser diodes

Introduction

CST Global is a world-leading volume manufacturer of laser diodes to ISO9001 standard. TopGaN is a global leader in GaN materials technology suitable for laser diode manufacture over a wide range of wavelengths, from u.v. to green. TopGaN and CST Global have combined resources to supply the latest GaN laser diode technology to the EDOCALD project facilitating the use of custom wavelengths which are not readily available commercially.

Overview on laser supply

For the first EDOCALD demonstrator TopGaN supplied a range of state-of-the art GaN lasers that covered a wide wavelength range from the u.v.,i.e., 395nm to the visible, i.e., 440nm in steps of ~5nm, to cover the bio-fluorescence window required for the dental study. CSTG fibre coupled the lasers to allow simple integration into a larger optical system, developed by FOCE and 2M, and provide optical beam delivery to the patient.

CSTG have implemented a laser diode manufacturing process with more precise wavelength control while at the same time improving manufacturability and yield. The 2nd generation chip designs enable more precise selection of the laser wavelength improved temperature stability.

In reporting period one CSTG produced lasers at 440nm for integration into the first demonstrator, and in the second period CSTG fabricated single wavelength 390 nm & 440 nm lasers for incorporation into the second demonstrator.

2.3.2 Description of laser manufacturing process

Gallium Nitride(GaN) lasers and subsystems are emerging devices with a wide range of applications in subsea and terrestrial communications, quantum technologies, display light sources and medical instrumentation.

CSTG have established a comprehensive design and chip manufacturing capability in these devices and the some of the potential subsystems and applications now being considered for spin-off exploitation activities require the development of a narrow wavelength, single frequency device.

These include plastic optical fibre communication systems to reduce the effects of chromatic dispersion, free space communications where narrow optical bandwidth operation at the one of the Fraunhofer lines is beneficial for background free operation, and narrow linewidth is a key aspect of laser cooling and measurement systems based on quantum effects.

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2.3.3 Laser active material

The active material used to manufacture the lasers, so called epitaxy, is based on Gallium Nitride/Aluminium Gallium Nitride/Indium Gallium Nitride layer structures. The emission wavelength of the device is determined by the design of the Quantum Well active region, with specific layer compositions and strain combinations.

A typical layer structure is given below, a so called Separate Confinement Heterostructure, along with the schematic of the model used in our simulation in order to optimise the and model the laser specifications. Epitaxy for this work was sourced from TopGan and from a third party.

Layer	Material	thickness	n	k
Insulator	siNx	450	1.9798	0.0003
P- metal	Pd	30	1.3885	3.19161
P-contact	GaN	40	2.4811	0
P -cladding	AlGaN	470	2.4513	0
P- waveguide	InGaN	50	2.4811	0
EBL	AlGaN	20	2.4025	0
top OCL	InGaN	70	2.5019	0
active reg.(averaged	InGaN	44.5	2.58	0
bottom OCL	InGaN	70	2.5019	0
n-waveguide	GaN	70	2.4811	0
n- cladding	AlGaN	1100	2.4513	0
Substrate	GaN	200	2.4811	0

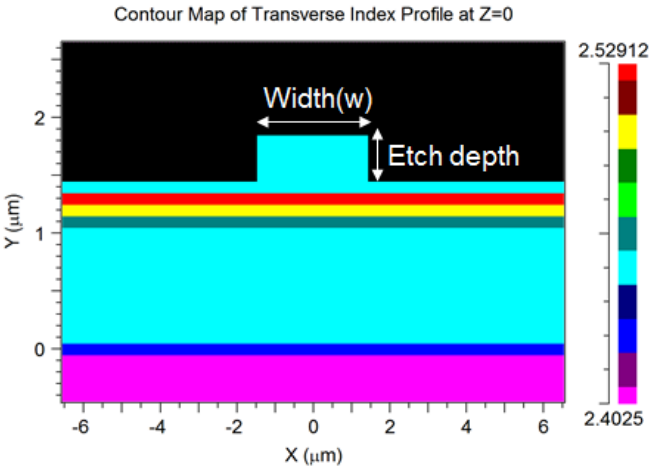

Figure 1: Epitaxial layer structure (thicknesses in nm) and cross section of ridge waveguide laser structure

2.3.4 Laser device design

A common method of improving the spectral quality of a semiconductor diode laser is implemented by the integration of periodic grating structure, which acts as an optical mode selection element (Cf Distributed Feedback Laser). Typically, in other compound semiconductor material systems, grating structures are integrated either above or below the laser active region and then 'buried' with additional epitaxial layers to form the laser waveguide. However, this is difficult in the GaN system since high Aluminium content waveguide layers tend to oxidise during the grating etch process, resulting in severe reliability issues.

CSTG have previously developed a simplified sidewall etched grating process on ridge waveguide laser devices, to avoid such reliability issues. This process has been implemented at a range of wavelengths and a range of structures and the main common advantage is that there is no need for a second stage epitaxial overgrowth. The main challenge in their manufacture is achieving the necessary refractive index modulation by changing the ridge waveguide geometry, whilst designing a grating that can be fabricated within the capability and tolerance of the fabrication toolsets.

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Fabrication of gratings for GaN lasers is especially challenging due to the short wavelengths involved. While a first order grating for a typical IR (eg1550 nm emission) laser will have a grating linewidth of 120 nm - this is reduced to 35 nm for a 440 nm laser – a feature size not practical with our current fabrication technology. Hence third order gratings with a feature size of 130 nm are feasible (still technologically difficult) but weaker in action than first order gratings.

Fig 2 illustrates how the coupling coefficient varies with grating duty cycle for various grating orders. In order to achieve coupling coefficients comparable with a first order grating, a high duty cycle should be chosen (~80%).

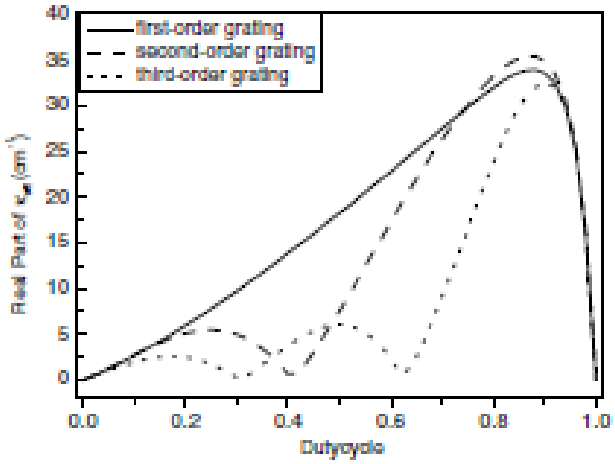

Fig 2. Coupling coefficient vs duty cycle for first, second and third order gratings as a function of duty cycle.

The coupling coefficient can be approximated by the following equation:

$$\kappa = k_0 (n_2 - n_1)\Gamma_{ov} \frac{\sin(\pi m \gamma)}{\pi m}$$

Where *m* is the grating order, k_0 is the wave vector, Γ is the overlap factor, n_1 & n_2 are the effective modal indices in the wide and narrow waveguide sections and γ is the grating duty cycle.

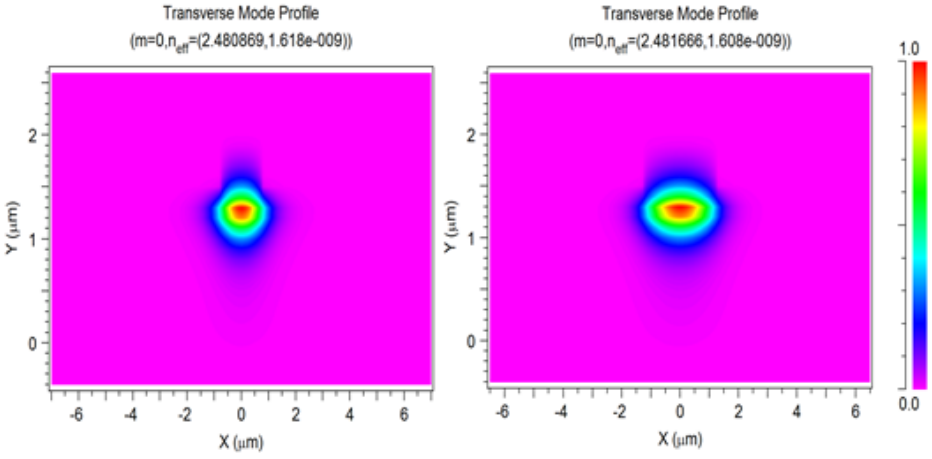

Fig 3: Mode profiles at 1.5um and 2.5um ridge waveguide widths

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The effective modal index refractive (n_{eff}) versus the ridge width(w) as shown on figure 1 can be calculated using a waveguide solver, in this case RSoft BeamProp. The depth of the ridge waveguide etch (again shown on figure 1) is also varied to check the dependence on this parameter. Typical mode distributions are shown in figure 3 showing the difference in modal shape for w=1.5 and 2.5. We can see that with associated mode field distribution change, there is a change in modal index from 2.480 to 2.481. The calculated n_{eff} is shown in figure 4 between 1um and 3um with etch depths ranging from 600nm to 700nm.

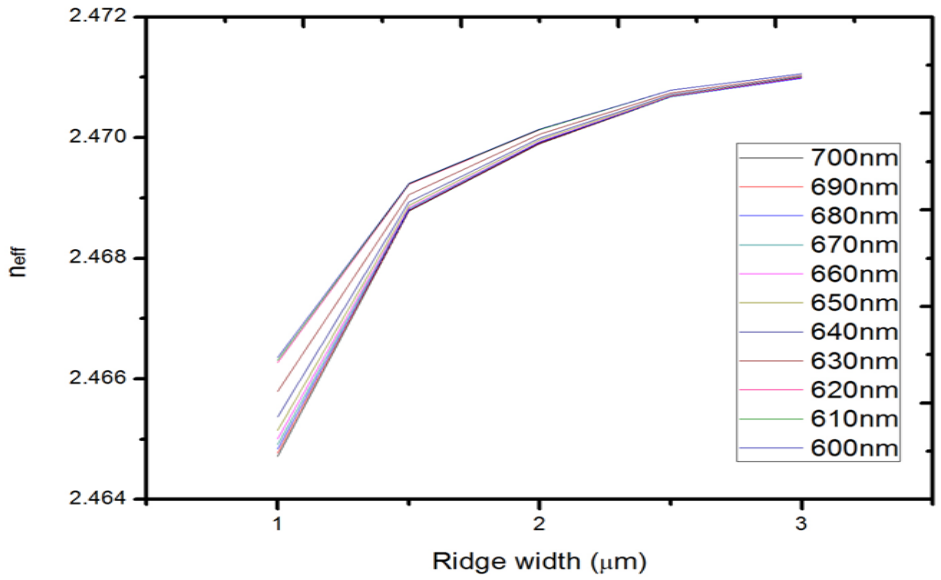

Figure 4: Variation in effective index with varying width (w) and etch depth.

We can see that, as expected, there is a trend of decreasing modal index with ridge width and also with increasing etch depth. The range of $\Delta n = (n_2 - n_1)$ for the preferred lateral grating waveguide widths of 1.5um and 2.5um is from 0.0015 to 0.002. Also clear from the figure is that the use of a 1um wide 'narrow' grating section would increase the Δn substantially to around 0.005. Using a delta n value of 0.002 we can estimate a maximum value of coupling coefficient of 6 cm^{-1} using a third order grating with a duty cycle of 80 %.

For a high single mode yield the product of κ and cavity length should be around 1.5 which leads to an optimal laser cavity length of 2.5 mm. For highest efficiencies and lowest threshold currents the optimal chip length for a typical GaN laser is around 500 to 700 µm and so some compromise will be expected between performance and single mode yield. To mitigate this we typically fabricate lasers with narrower grating widths albeit with the added fabrication risk associated with narrow mesa formation and contact window opening.

Given the uncertainty in the refractive index data, epitaxial structure and manufacturing process bias, it is always prudent to include a wide range of device designs in terms of length and waveguide widths on a wafer fabrication iteration. Final chip designs therefore typically include grating widths of 1 µm/2µm & 1.5 µm/2.5 µm with cavity lengths of 500 µm, 1000 µm & 1500 µm.

Furthermore, given the associated uncertainly in the modal index of the waveguide, a range of 5 grating pitches are usually included to ensure that at least one pitch will give a Bragg

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wavelength which overlaps with the gain spectrum of the laser material. All other aspects of the chip design and processing are similar to the basic ridge waveguide GaN lasers currently made by CSTG to reduce the fabrication and design risk as much as possible.

2.3.5 Fabrication

The laser fabrication process is based on an existing standard CSTG route with the additional critical steps of patterning and etching of the fine pitch grating. Patterning was carried out by electron beam lithography (EBL) using the VB6 system at the University of Glasgow.

The write resolution used was 1nm, enabling the accurate targeting of the emission wavelength. Fig 5 shows SEM and AFM images of the patterned ZEP520 resist and reveal a grating duty cycle of approx 40 % - less than the design target of 70 % due to over-exposure during the EBL.

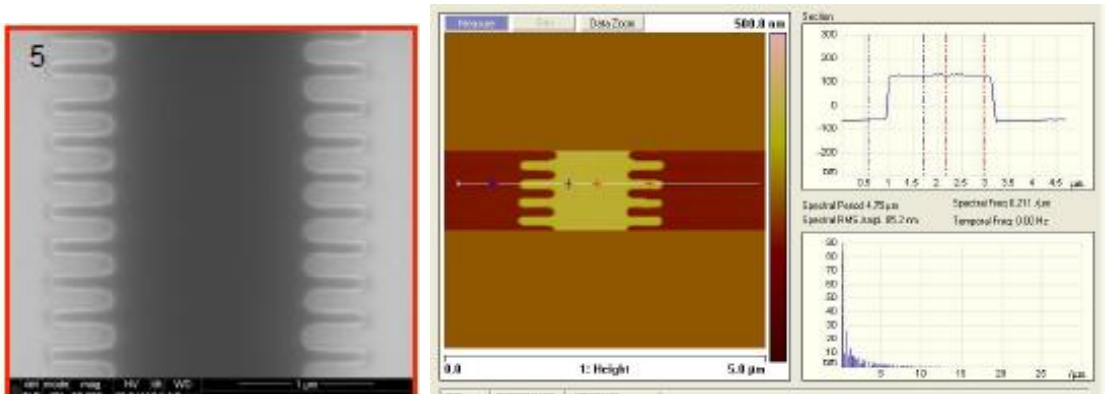

Fig 5: SEM & AFM images of the patterned resist.

Reactive Ion etching was used to transfer the pattern from the resist into an SiO_2 hard mask. Fig. 6 shows the grating defined on oxide after etching and resist removal. At this stage the duty cycle has increased back to around 70 % due to the process bias during etching. Fortunately this is close to the design value and demonstrates how important an understanding of process bias becomes at these feature sizes.

Inductively coupled plasma (ICP) etching on an STS Multiplex tool is used to form the deep lateral grating. In order to achieve the high aspect ratio and small feature size required of the grating, a highly smooth and vertical ICP process previously developed at CSTG is used. Post grating etch, the wafer processing follows a standard, qualified CSTG device fabrication route. A Cl2/N2 based ICP etch process with 300 W platen and 600 W coil power produced the vertical and smooth etch profile required for good grating performance. Fig. 6 shows an electron micrograph of an etched grating. Electrical contacting was achieved using Pd/Au and SiO2 as an insulator for contact definition on the Mg doped p-GaN cap layer.

Finally the samples were thinned by mechanical polishing and the devices were cleaved to 1.0 mm cavity lengths with both front and back facets left uncoated.

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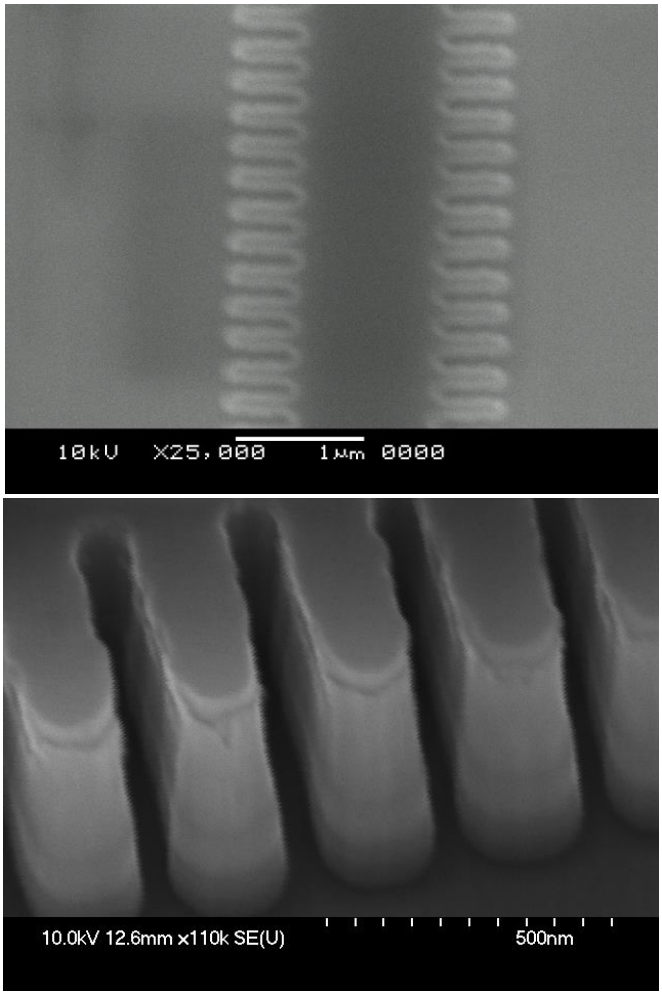

Fig 6. SEM image of grating in hard mask after hard mask etch and resist removal

2.3.6 Typical device results

Lasers were characterised under pulsed drive conditions. LIV measurements were carried out using a Keithley automatic test system. An Ocean Optics spectrometer with 0.1 nm resolution was used for spectral measurements.

Typical output spectra at room temperature are given in Figure 7 below. The red spectrum is a reference measurement from a standard GaN ridge waveguide laser without grating coupling. It is clear that the mode spectrum is complex and there are competing optical modes which will degrade any precise optical measurement technique.

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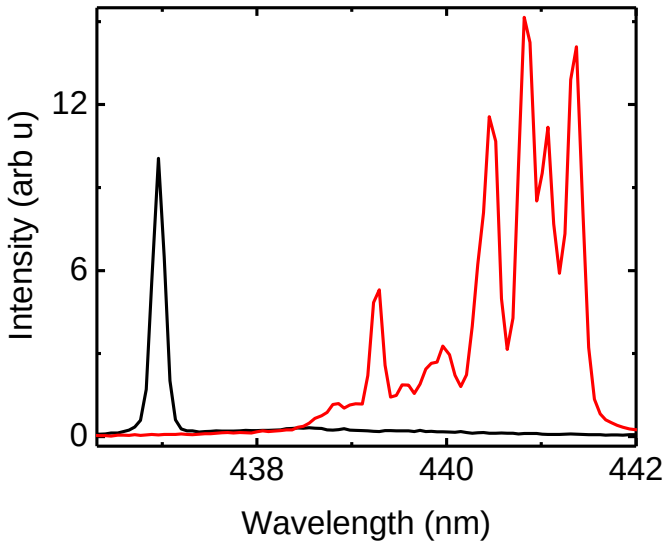

Fig 7. Spectral measurement of grating coupled and non-grating coupled ridge waveguide GaN lasers

The black spectrum is from a 3rd order grating coupled GaN ridge waveguide, DFB laser diode. The device output is wavelength shifted to sub 440nm by deliberate design of the grating geometry. In addition, the optical output exhibits 22dB side-mode suppression (which we believe is a record for a Gallium Nitride blue laser), and narrower optical line-width compared to the non-grating coupled laser. The mode is stable up to a drive current of 600mA, which corresponds to the high end of any operating range.

2.3.7 Conclusions

- CSTG have supplied a range of GaN laser wavelengths fabricated from material supplied by both TopGan and 3rd party epitaxial suppliers.
- 'Non-standard' wavelength diode lasers have been demonstrated and supplied at 390nm and 440nm.
- Diode lasers have been packaged and fibre coupled for integration in both EDOCALD demonstrator systems.
- The initial devices were fabricated using a basic Ridge Waveguide chip fabrication process which exhibited acceptable specification.
- For the second generation devices, CSTG have implemented an enhanced grating coupled design and manufacturing process.
- The grating coupled process offers the benefits of :

    - improved single-mode spectral performance and stability
    - higher yield based on wavelength selection
    - a low cost route to stabilised, temperature tunable lasers

- The enhanced specification offers potential for exploitation outside the EDOCALD application in several niche applications.

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2.3.8 The test systems

Four test systems were designed and developed during the course of the project, these systems were subsequently used to perform the measurements on standard references, cell cultures, in-vivo tissue and eventually clinically. These systems were designated:

- Demonstrator 1A,
- Demonstrator 1B,
- Demonstrator1C, prototype for WP7 & 8
- LEDs versus LASERs (LVL)

Two of the systems were developed for clinical use, namely Demonstrator 1B and Demonstrator 1C. These two systems include safety mechanisms to prevent excessive exposure to the violet and ultra-violet light excitation generated by the systems.

2.3.9 Demonstrator 1A

Demonstrator 1A was developed as an initial platform to allow laboratory evaluations that would help in the choice of architecture and components for the systems that would be used clinically on patients. It incorporated light sources of wavelengths that would be suitable for stimulating auto-fluorescence in oral tissue types (325nm, 365nm, 385nm, 400nm, 405nm, 415nm, 420nm, 425nm, 430nm, 440nm, 625nm) and a broad band white light source for calibration purposes. Where possible these wavelengths were generated using semiconductor LASERs, this was the case for the 400nm, 405nm, 415nm, 420nm, 425nm and 430nm wavelengths. Other wavelengths were generated using Light Emitting Diodes (LEDs), and in the case of the broad band white light a halogen lamp was used. A system of drivers for the LASERs and the white light source was developed incorporating a microcontroller and USB interface was developed to ensure that the light sources were controlled in a way suitable for the required auto-fluorescence measurements. The LEDs were driven by a separate USB connected controller integrated into the system. This approach provided a flexible and effective means of controlling the excitation system.

Figure 1: Demonstrator 1A (Black box with blue LED)

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The auto fluorescence detector was implemented using a high quality spectrometer incorporating a thermally controlled sensor operating at -10˚C, providing high quality, low noise spectral measurements.

The light from the various LED sources was combined using dichroic mirrors and coupled into an optical fiber, while the light from the other sources was directly coupled in to optical fibers. Subsequently the light from the optical fibers was merged using fiber optic combiners into a single fiber bundle that was fed into one leg of the bifurcated probe, which guided the light to the samples.

The optical power of the excitation sources at the probe tip achieved is shown in Table 1.

Wavelength	Optical Power at Probe Tip
365nm	265uW
385nm	550uW
400nm	342uW
405nm	363uW
415nm	264uW
420nm	417uW
425nm	352uW
430nm	200uW
625nm	329uW

Table 1: Optical power at Demonstrator 1A probe tip

Auto-fluorescent and reflected light from the excited tissue sample was guide along the other leg of the bifurcated probe via an optical long pass filter to the spectrometer, the filter preventing artefacts in the spectra measurements caused by the higher order diffraction components of the relatively high power excitation sources.

Software was developed to run on system computer running the Windows operating system. Two software applications were developed, one for the laboratory use case, the other a for the clinical use case (even though this system was not going to be used clinically on patients). The two application programs run on a common software library developed to control the Light Source and Capture Unit, providing control over the intensity and sequencing of the light sources, the capturing of the spectral measurements. The two software applications differed in that the clinical software included imaging functionality for images from an intra-oral camera, and a database system allowing the tracking of images, changes in those images and spectral measurements using the Light Source and Capture unit. A single click with a mouse or the foot-operated switch is sufficient to run a sequence of excitation exposure and spectral response capture for each of the light sources, providing a quick, easy to use, reliable and repeatable measurement set.

2.3.10 Demonstrators 1B and 1C

Demonstrators 1B and 1C were developed primarily for clinical use on patients, the physical size of the Light Source and Capture unit was reduced considerably (from 525x417x430 mm to 350x260x120 mm, a volume reduction of more than 8:1), making them suitable for transport between and use in dental consulting rooms. Demonstrator 1B and 1C were built to

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be as identical as possible to ensure ease of use for the clinicians and to provide consistent and comparable data sets for analysis.

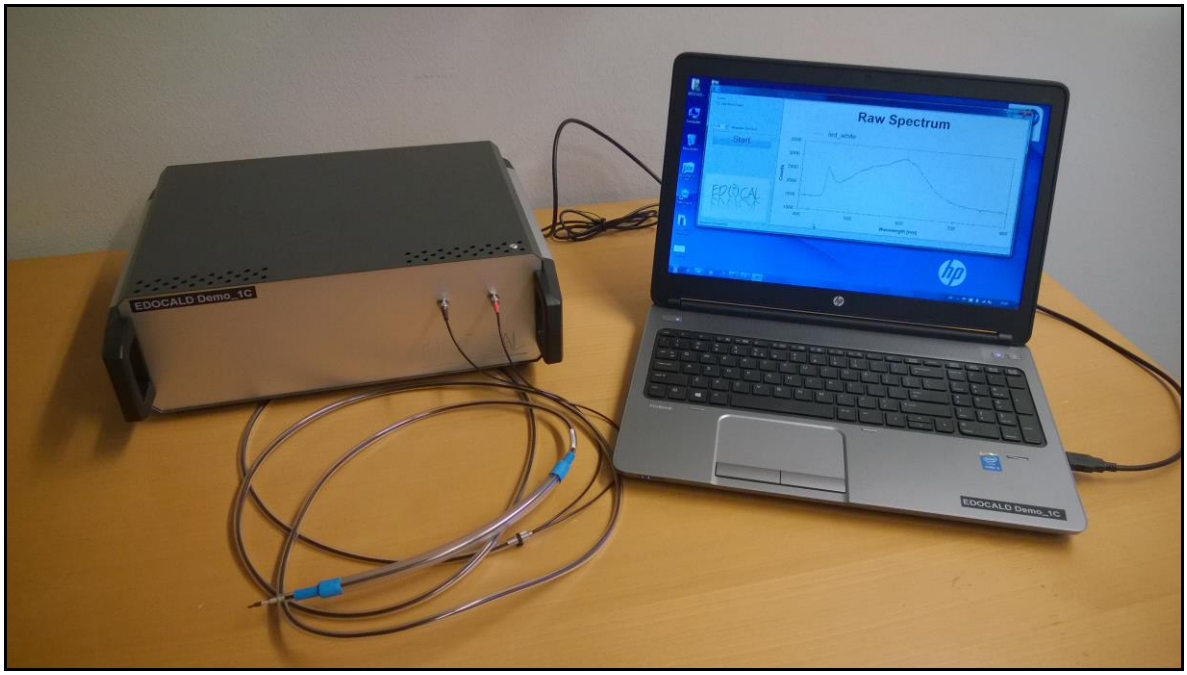

Figure 2: Demonstrator 1C

Smaller volume was made possible by optimizing the system components:

- Alternative LASER couplers and combiners delivered not only a decrease in size but also an improvement in efficiency,
- An alternative Spectrometer provided a significant reduction in size, power consumption and cost, while maintaining sufficient signal performance,
- Replacement of the halogen broadband white light source by a white light LED provided a size, power consumption and cost reduction,
- New driver electronics was developed based on the concept used in the Demonstrator 1A, but this time including driving of the LEDs sources (including the white light) provided a significant reduction in size and power consumption while adding safety features making the system suitable for clinical patient use.

Excitation light sources of 365nm, 385nm, 400nm, 405nm, 415nm, 420nm and 425nm were incorporated in Demonstrators 1B and 1C alongside the broadband white light LED. In these systems the 400nm, 405nm, 415nm, 420nm and 425nm wavelengths were implemented with semiconductor LASERs while the other light sources were implemented using LEDs.

The optical power of the excitation sources at the probe tip achieved is shown in Table 1.

Wavelength	Optical Power at Probe Tip
365nm	1020µW
385nm	1024µW
400nm	1011µW
405nm	1007µW
415nm	1075µW

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420nm	1076µW
425nm	1012µW

Table 2: Optical power at Demonstrator 1C probe tip

2.3.11 LEDs versus LASERs (LVL)

The LVL system was created to provide a means of comparing the performance of LED and LASER light sources as excitation while measuring auto-fluorescence from standard references and cell culture samples. It was built on the platform developed for Demonstrators 1B and 1C, however it was designed to be used in conjunction with Demonstrator 1A (as shown in Figure 3: LVL is used in conjunction with Demonstrator 1A), the high quality spectrometer in Demonstrator 1A being used for the spectral capture. Two sets of excitation wavelengths were incorporated: 405nm and 440nm. For each of these wavelengths both a semiconductor LASER diode and a LED were implemented.

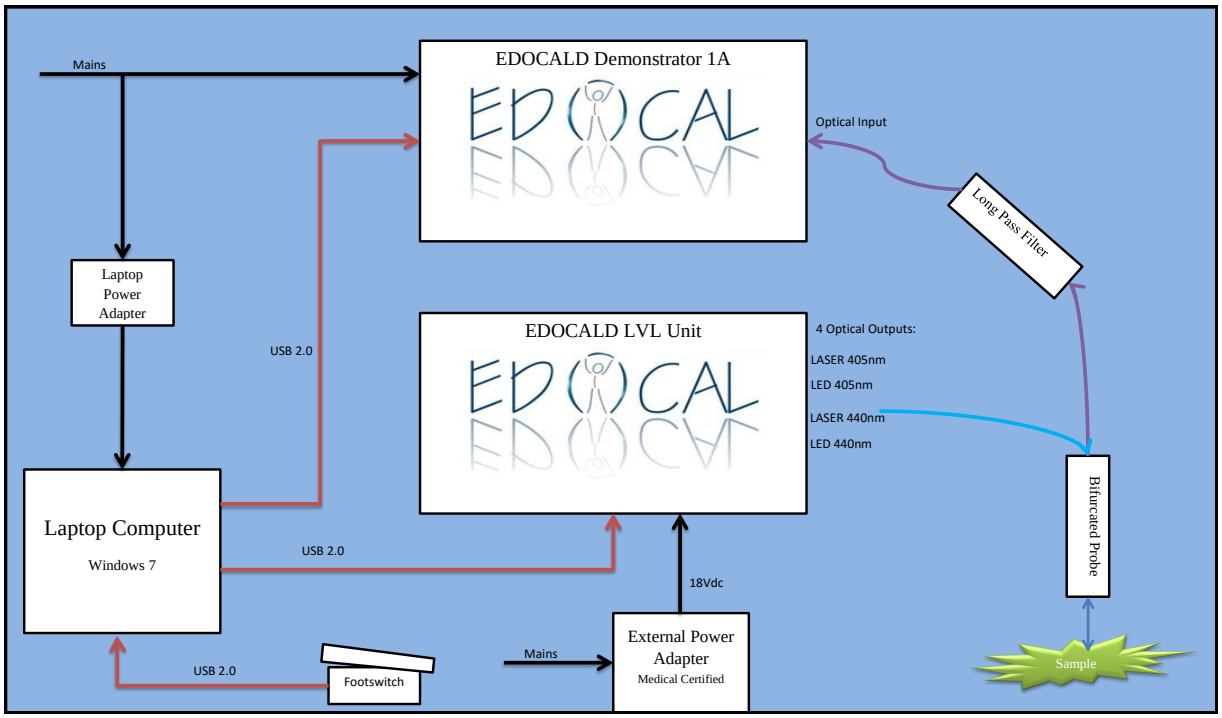

Figure 3: LVL is used in conjunction with Demonstrator 1A

The optical powers achieved at the LVL outputs was determined by the powers achieved by the LASERs, the LED powers were adjusted accordingly.

Type	Wavelength	Optical Power at Output
LED	405nm	1150µW
LASER	405nm	1150µW
LED	440nm	228µW
LASER	440nm	228µW

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2.4 The image database

Currently a dentist has no way to track a lesion or suspect area. The imaging system that has been developed as part of this project uses triangulation to position the new image on top of the old one making it clear whether the lesion is increasing or decreasing in size and or colour or not changing at all.

Even though such a tool would seem to be a logical requirement for a dentist/dental surgeon, no such tool exists today. This is particularly important tool in group practices when patients can be seen by different dentists on each visit. The imaging system provides objective information allowing the dentist to track suspect lesions in an objective way.

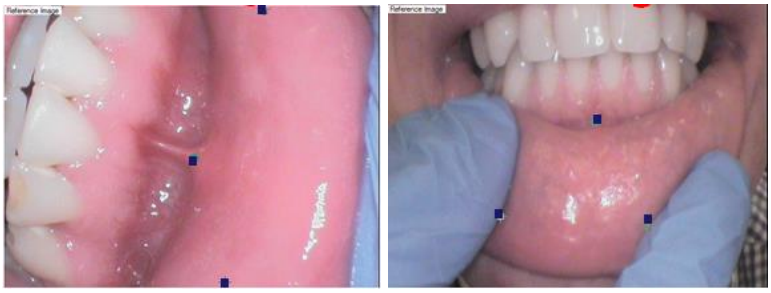
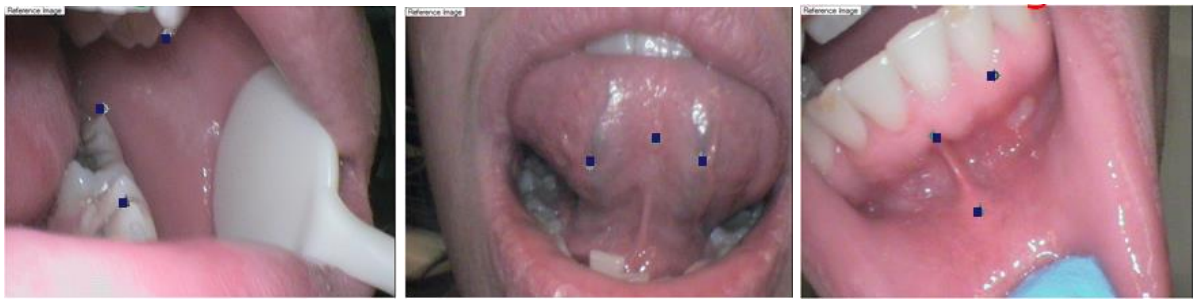


Fig.1 – Examples of bad choices of reference points



The imaging database is a subset of the information, containing only the images allowing the professional to record and track suspicious areas in the patient. The algorithms used to perform the triangulation and superimposition of the images is described under the Spatial Reference Algorithms.

The aim is to reduce the number of patients that are being referred to dental surgeons unnecessarily by first having the dentist track the lesion at the bi-annual dental checkups, including extra checks up to examine suspect areas if the dentist thinks it is required. It appears that there is no standard practice defining when patients get referred and when they do not. This is very dentist dependent.

The system has a second benefit of having patients who may otherwise not be referred on time because the dentist doubts and decides to wait be referred sooner. Both cases have the potential to reduce health care costs. We have not looked at this in detail yet because it is a recent conclusion and an addition to the project.

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3 WP3 Pre-clinical testing

EDOCALD Work Package 3 (WP3) Objectives

The objectives of the deliverables in this report and part of work package 3 (WP3) are to characterise the demonstrator device, to validate the wavelength suitability for illumination and fluorescence detection in the oral cavity, to provide with proof of efficacy of the device.

3.1.1 Task 3.1 (D3.1): Device characterisation

Device characterisation was carried out in the department's optical radiation calibration laboratory. This is an accredited laboratory (accreditation from United Kingdom Accreditation Service, UKAS). Measurements were performed in a temperature controlled environment with calibrations traceable to national measurement laboratories. The principle findings were

1. Satisfactory risk/safety assessments (UV/visible radiation and electrical) have been carried out.
2. Characterisation of the optical radiation. The results include the shape of the emission spectrum, peak wavelength and full width half maximum (FWHM)
3. Characterisation of the radiant power and risk assessment of the LVL in addition to EDOCALD1A.

3.1.2 Task 3.2 (D3.3): Wavelength suitability

Autofluorescence (AF) is a natural phenomenon where cells and tissues emit light after having absorbed it. Human skin and oral mucosa have similar structure in that they are stratified, with an epithelium separated from an underlying connective tissue by a basement membrane. Skin consists of orthokeratinized epithelium, whereas the oral cavity also contains parakeratinized areas and non-keratinized areas. There are differences in the connective layer/appendages also, in that the oral cavity does not have sweat glands or hair, but does have salivary glands and some sebaceous glands [1].

Fluorophores that contribute to AF
Endogenous fluorophores that contribute to tissue AF absorb mostly in the 315-450 (UVA/Violet/Blue) waveband [2, 3, 4, 5]. These molecules include:

Amino acids with aromatic side-chains (tryptophan, tyrosine, phenylalanine) λex: 270-300 nm; NAD(P)H λex: 340-350 nm; Flavin λex: 380-490 nm; Lipofuscin λex: 410-470 nm; Melanin λex: 340-400 nm; Retinol λex: 325-335 nm; Porphyrin λex: 396-410 nm; Riboflavin λex: 380-460 nm. In the skin, there are contributions from other molecules including carotene, cholecalciferol, folic acid and pyridoxine. In skin, the largest contribution to AF probably comes from NAD(P)H and flavins, and also collagen and elastin. In other tissues eg. brain, fluorophores such as lipofuscin may play a bigger role [6].

Ageing, disease and oxidation change the AF of skin from both the upper and deeper layers. In the literature, a number of discriminatory wavelengths have been identified. It is clear that

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multiple excitation wavelengths seem to be necessary for the development of optimal spectroscopic algorithms for differential diagnosis [7].

Validation of EDOCALD demonstrator using AF of inner forearm.

Readings were obtained from five subjects. The EDOCALD device is fitted with a single 400 µm fibre and the data were not adjusted for skin colour. The signals were corrected for instrument response (dark signal) and power of the source and represent the fluorescence intensity (FI) as determined by an Allen correction of the curve ('skimming' technique). In this small pilot, the wavelengths that most effectively generated a raw AF signal after correcting for power from normal, forearm skin were:
385 (4/5)/405(5/5)/420(5/5)/ > 415/425/430 > 400 > 365 (5/5)
The figures in brackets indicate that eg. The signal was lowest in 5 of 5 subjects for the 365 nm excitation wavelength.
From previous experience, we are confident that the Allen corrected peak heights are a good estimate of AF from the site. The skin-enabled fluorimeter is not suitable for measurements inside the oral cavity due to the size of the probe. Nor is it suitable for the accurate measurement of small lesions such as cancers. The output in the violet/UVA range is also low in comparison to the EDOCALD device.

The wavelengths that most effectively generated an AF signal from normal, inner lip were:
385 (5/5) > 405/420 > 365/415/425 > 430 >400 (3/4)
Generally, the overall AF pattern was similar to skin. Compared to skin, AF was a little lower for excitation wavelengths of 385 nm or longer, and a little higher at 365 nm.

The analysis was performed on three repeat samples from four individuals to give an estimate of precision of the measurements. The results suggest that the positioning of the probe has a bearing on the results obtained.

Three repeat samples were taken from three sites: inner forearm, inner lip and outer lip. As before, the probe was removed from the site and replaced between the readings. The spectra were corrected for the dark signal and were power corrected. Because the emission spectra were truncated: that is, the baseline at the right and left hand side of the emission peak could not be determined in all cases, the fluorescence intensity was calculated two separate ways. In the first case (without baseline) Prism calculated the area under the curve using the x-axis as the baseline. In the second case (with baseline), an Allen correction (skim) of the emission was taken as described above.

Conclusion

1. AF measurements of the normal inner forearm from the EDOCALD device are in agreement with previous data obtained by the Photobiology Unit using a skin enabled fluorimeter.
2. AF data obtained from the normal inner and outer lip is in good agreement with that obtained from normal skin, therefore the wavelength range used in the EDOCALD device should be suitable for measurements in the oral cavity despite the differences in tissue structure.
3. A source < 365 nm may also be useful in discriminatory studies.

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4. The excitation/emission profile suggests that in the case of both the forearm and the inner lip the main contribution to AF is from structural components.

3.1.3 Task 3.3 (D3.2): Device Validation: Proof of Efficacy

In Task 3.3 we outlined the contribution of different fluorophores to tissue AF (AF). We summarised how factors that influence AF can be broadly divided into two groups: structural and metabolic. We also described how both of these can be influenced by age, disease, etc. especially if the tissue is highly ordered or stratified like the skin, and thus multiple excitation wavelengths were likely to be necessary for the development of optimal algorithms for differential diagnosis [7].

Validation of EDOCALD demonstrator

We concluded that the wavelength range used in the EDOCALD device should be suitable for measurements in the oral cavity despite the differences in tissue structure from the skin, and that the excitation/emission profile suggested that in the case of both the forearm and the inner lip the main contribution to AF might be from structural components.

Metabolic markers: NADH & FAD

Only the reduced forms of adenine nucleotides (NADPH/NADH), and flavin nucleotides (FAD) fluoresce.

NADH dominates cellular fluorescence over NADPH because mammalian cells have about 5 fold more NADH. NADH can be excited with wavelengths between 330-370 nm, and the emission measured > 440 nm. The intrinsic fluorescence of NADH has been used in non-invasive diagnosis of colon, cervical, oesophageal, lung, bladder and breast cancers, because cancer cells have an increased metabolic demand compared to normal cells. The Warburg effect also mean that metabolism shifts from efficient aerobic metabolism to less efficient anaerobic glycolysis; when glycolysis is upregulated, NAD+ is reduced to NADH [8].

Solutions of reduced β-nicotinamide adenine dinucleotide 2’ phosphate (Sigma-Aldrich Ltd, UK) were prepared in Earle’s balanced salt solution (EBSS). Solutions were pipetted (100 µl) into the wells of a black-walled µclear 96 well plate (Greiner Bio-One, UK). The solutions were freshly prepared before each experiment. The EDOCALD 1A probe was held in place by a clamp and solutions were irradiated from below, through the base of the plate. Emission spectra were corrected for the dark signal, and output power. The curves were integrated over the wavelength range > 470 nm.

EBSS base solution shows minimal detection of fluorescence emission at wavelengths >470 nm. NADP(H) was detected in a dose-dependent fashion, with maximum emission obtained with the 385 & 365 nm LEDs. This is consistent with the absorption spectrum of NADP(H), which has a maximum at approximately 340 nm. Light emission during excitation with the 365 nm LED was highest at the low doses of NADP(H), but quickly saturated; whereas emission upon activation with the 385 nm LED tended to increase linearly. This is consistent with the absorption spectrum, where 365 nm is very close to the absorption maximum, but 385 nm is far down on the shoulder of the absorption peak. Minimal fluorescence was

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detected at 400/405 nm at the highest dose of NADP(H), and this decreased as the excitation wavelength increased. Again, this is consistent with the absorption spectrum where there is minimal absorption at wavelengths > 400nm. Reabsorbtion or inner filter effect may account for the lower maximum emission at 365 nm excitation compared to 385 nm excitation.

Metabolic markers: Use of Cultured Cells

It was anticipated that the AF generated from monolayer cell cultures would be low, and an experimental set-up had to be determined. It was found that placing the cells into the wells of a black-walled µclear 96 well plate (Greiner Bio-One, UK) was the most reproducible solution. The EDOCALD 1A probe was held in place by a clamp and solutions were irradiated from below, through the base of the plate. The lid of the plate was left off during readings, and the readings were taken in darkness. Emission spectra were corrected for the dark signal, and output power. The curves were integrated over the wavelength range > 470 nm. The redox ratio was calculated by dividing the FAD fluorescence by the sum of the NADH and FAD fluorescence [9].

HaCaT cells were seeded onto the plates 18-24 hours before the initiation of the experiments, so that they would achieve confluence.

Detection of PpIX

HaCaT cells were treated for 1 hour with 2.5 mM methyl aminolaevulinic acid, which is a prodrug of PpIX. EDCALD 1A was able to detect PpIX fluorescence in the HaCaT cells. There were two peak maxima: one at 633-636 nm and a second at 705 nm.

Effect of cellular stress

Conditions which decreased cellular metabolic activity caused an increase in the redox ratio. The changes in redox ratio seemed to be mainly influenced by a decrease in the NADH signal, which decreased by 34.5 ± 3.1%, compared to the FAD signal which decreased by 14.0 ± 6.7%. The results are consistent with previously reported results where cells have been treated with chemotherapeutic agents [9].

Effect of modifiers of mitochondrial metabolism

FCCP is a mitochondrial uncoupler, which maximizes respiration and causes oxidation of the NADH pool. This should result in a decrease in AF, especially at 365 nm. Conversely rotenone should increase AF by promoting the formation of NADH. Although the trends we saw in U937 cells were consistent, they were not as marked as has been reported by others [10, 11], especially the results with rotenone. Findings also appeared to be cell type dependent as changes were very difficult to detect in HaCaT cells. When the cells were incubated with MTT (a marker of mitochondrial function), we could discern changes in the HaCaT cells via the light microscope, especially for the FCCP-treated samples, which would be consistent with what was detected by EDOCALD 1A.

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Structural markers: Use of 3D model systems

Monolayer cell cultures can provide only limited information for a device that can selectively measure signals from different tissue depths because of their thickness. Therefore, we examined the efficacy of EDOCALD 1A in a pilot in-vivo study and in 3D model systems.

Data was obtained from a skin type IV individual with dermatitis on the back of the hand. 2/3 readings were taken from the dermatitis lesion and from surrounding uninvolved skin at the same site. The redox ratio was calculated by dividing the intensity at 430 nm by the sum of the intensities at 365 nm and 430 nm.

The results show a decrease in AF from the lesion especially at excitation wavelengths > 365 nm. The difference between normal and lesional skin at 365 nm was minimal. AF generated seemed to show a greater decrease from normal as the excitation wavelength increased. The redox ratio was decreased in the lesional skin (0.461 ± 0.04) compared to normal skin (0.541 ± 0.06).

We did not biopsy our sample site. An explanation for the decreased AF observed at longer excitation wavelengths could be due to oedema and spongiosis. The relatively little change at 365 nm could be consistent with little or no change in epidermal thickness at the surface [13].
We also showed a decrease in the redox ratio of the lesions, due to changes in fluorescence intensity mainly at 430 nm (ex), but also at 365 nm (ex). Evidence to date is suggestive that cancer cells in experimental culture systems demonstrate a higher redox ratio than healthy cells due to increased cellular NADH [14] thought to reflect an increased metabolic rate. This could partly explain why systems relying on one or a few excitation wavelengths have difficulty distinguishing benign, inflammatory conditions from neoplasia.

Scar tissue

Data was obtained from a skin type II individual with scarring as a result of a scald on the back of the hand. The results show a decrease in AF from the lesion especially at excitation wavelengths < 400 nm. The difference between normal and lesional skin at longer excitation wavelengths was minimal. In contrast to the data obtained with the dermatitis sample, the redox ratio was increased in the lesional skin (0.429 ± 0.06) compared to normal skin (0.389 ± 0.04), mainly due to differences in fluorescence intensity at 365 nm.

The data from each experimental run show that in 2/3 runs the readings were very consistent. For 1/3 runs, the trend remained the same, but there was a difference in fluorescence intensity. For these experiments, the probe was held by the operator and was not clamped in place. Despite the differences in intensity, AF was always lower compared to the surrounding skin, thus the device could be useful for monitoring an individual (using them as their own control).
Scars caused by physical (mechanical) trauma from the lower leg of two individuals were also assessed. Again the data show decreased AF from the lesion. The redox ratios were: scar 1: normal = 0.368 ±0.03; lesion = 0.371 ±0.003. scar 2: normal = 0.388 ±0.006; lesion = . 0.358 ±0.02. The redox ratios in these injuries was the same or lower than from the surrounding normal skin, whereas the scald had a higher redox ratio. Hot liquid scalds tend

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to cause superficial damage in contrast to the deeper tissue damage that would have been sustained in the mechanical injury scars. However, without biopsying the examples we cannot conclude that the differences we observed are related to structural damage or metabolism.

3D Model system

We ordered two 3D cell cultures: Melanoderm Model (MEL-300-FT-C1) to simulate Normal Skin and Melanoma Model (MLNM-A375-FT) to simulate Cancerous Skin. Data was obtained from corrected fluorescence measurements by shining the probe from above the glass bottomed dish onto the top of the 3D culture without membrane at the base.
There is a difference observed between the AF signals for the Melanoderma and Melanoma models with Melanoma model probably generated higher levels of advanced glycated endproducts in response to 250mM ribose. The highest signals have been generated by the 250mM ribose set for both the cutlures whilst 5 and 30mM signals were very close to the control signals with no ribose treatment. The control 0 day data is relatively quite low and on the right graph, the microporous membrane is showing high fluorescence signal at all exciattion wavelengths.

Biopsy Samples
The normal counterpart (non-lesion) biopsy was collected within 3-5 cm of the lesion area, from where the lesion biopsy was collected. The Glasgow-based supplier, 'Tissue Solutions' got the ethical approval for us. The samples after measurement of AF were discarded appropriately.

Biopsy Sample results
The excitation wavelength of 365nm and emission at 420-550nm was used to account for the collagenase digestible collagen cross links (CDCCL). Also the exciation wavelength of 420nm with the emission at 480-550nm was used to account for the Elastin cross links (ECL). The Healthy skin data represented is the data from innerarm of Healthy volunteers generated by us earlier during the study and not from a biopsy sample. The results clearly show that spectra obtained from both lesional and non-lesional areas of psoriatic patients have similar spectra between themselves.

EDOCAL1A with LVL
Lasers vs LEDs device was tested on: (a) Standards (liquid standard Coproporphyrin, and solid fluorescence standards Ovalene & Compound610), (b) 10 healthy volunteers (3 sites: Innerarm, Innerlip and Outerlip), (c) Psoriatic lesional and Non-lesional Biopsy samples procured from Tissue Solutions. Healthy human volunteers were from a mixed age range. The raw data from each of the four sources was corrected by subtracting the corresponding Dark from source data and then power correct with power outputs. The three different sites namely, innerarm, innerlip and outerlip were chosen and the probe was covered with camera sleeve. Testing the lesional and non-lesional samples from the psoriasis patients using the Lasers vs LEDs device reported similar peak shapes from the 405nm and 440nm Laser and LED. The probe was covered with the camera sleeves for testing the tissue samples. The standards were scanned as well before and after the tissue samples were tested. The raw data was processed in a similar format.

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Analysing all the data generated from the Lasers vs LEDs device, it was been suggested that the data from 440nm Laser and the LEDs overlap beyond the 465nm uptil 785nm. The signal levels are similar with laser and LED for both 405nm and 440nm.
Therefore, a filter on the LEDs would be installed in the LVL device and it shall be further tested on healthy volunteers to compare the Laser vs LEDs.

Summary

1. The mitochondrial/metabolic activity of the cultured cells was functional under the experimental conditions used. Cells were able to convert methyl aminolaevulinic acid to PpIX, and a change to mitochondrial activity caused by the modifiers rotenone and FCCP was confirmed by changes in the reduction of MTT. An increased intensity of MTT staining was observed with FCCP, whereas incubation with rotenone decreased staining intensity or left it unchanged.
2. EDOCALD 1A was able to detect changes in AF of cultured cells; however the AF signal was very low. The general trend was that AF tended to increase or remain the same in the presence of rotenone, but decrease in the presence of FCCP, menadione and incubating the cells in salt solution.
3. The redox ratio is unlikely to be as straightforward as described in the literature, especially in tissues, where many metabolic and structural components will contribute to the emission detected.
4. EDOCALD 1A was also able to distinguish normal skin from lesional skin. AF was always lower in the lesional skin, but there were differences between scar tissue and dermatitis. In dermatitis and one of the scars there was greater loss of signal at longer excitation wavelengths. The scald and second scar showed a greater comparative loss at shorter excitation wavelengths. Repeated testing of the scald over several days demonstrated a consistent trend. This suggests the device will be useful for individual monitoring of AF.

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3.2 WP4 Data analysis and Algorithms

Summary

The EDOCALD Oral Lesion Detection system uses the cancer detection algorithms developed in the EDOCAL project [1]. In addition the system provides additional features to permit different types of white light correction, different types of lesion to contra lateral tissue comparisons, multiple single and double ratio calculations at selectable wavelengths or wavelength ranges, additional normalization options in single and multiple sources and Principal Component Analysis (PCA). Furthermore the algorithms provide Excitation Emission Matrix (EEM) output for visual analysis of spectra recorded. Finally peak fitting algorithms have been implemented to separate different spectral components. The algorithms have been applied to the available dataset taken from 61 sites in 48 patients where for each patient multiple lesion and non-lesion spectra were collected (typically 3 each) for 7 excitation wavelengths resulting in around 2200 spectra. Analysis resulted in high sensitivity and specificity for malignant tissue detection for the small dataset with malignant tissue (5 sites from 2 patients) that was available (as in [2]). However the detection of potentially malignant tissue is much more difficult; up to 2/3rd could be detected albeit that a similar fraction or probably less ([3], [4]) is known to develop into cancer eventually so that this result may be understood. The spectra from the malignant sites are dominated by emission peaks at 635 and 705 nm due to Protoporphyrine-9 (PpIX) fluorescence and this dominates both the (double) ratio and PCA analysis; it is not known whether this is due to the tissue emission itself or other sources such as bacterial infection. In order to compensate for the spectral components due to PpIX fluorescence a peak fitting algorithm was added to the analysis to separate these components from the rest of the spectra. This has shown that all the spectral features in the wavelength range beyond 625 nm are dominated by this PpIX or related fluorescence.

Algorithm

The algorithm includes the processing that was used in the first EDOCAL project developed by the AMC. This consists of white light correction of measured spectra, spectrum smoothing over 6 nm and normalization in integrated spectrum intensity from 480 to 680 nm followed by single ratio calculations between emission in the 550-570 nm range and in the 630-650 nm range resulting in a single ratio per excitation wavelength. Double ratio calculations were obtained by dividing these single ratios for different wavelength combinations and these provided the best results in the EDOCAL project. Functionality was added for automatic optimization of single and double ratio calculations for best sensitivity and specificity and to select the optimum combination to use for double ratio calculations. Excitation Emission Matrices (EEM) are available for visual inspection.

Options were added to the EDOCAL algorithm for spectrum normalization in average power per wavelength source, average overall power in a measurement set and average power per measurement site. White light correction factors can be scaled in the average white light reflection spectra. The fluorescence spectra can be normalized in spectra taken from non-lesion sites, usually at contra-lateral sites from lesions sampled in the oral cavity. The spectra can be normalized in the non-lesion spectra from the same patient or in the non-lesion spectra of the entire data set. The normalization can be performed per excitation

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wavelength individually or in a selected excitation wavelength. Principal Component Analysis (PCA) was added to the analysis that can be executed per excitation wavelength source or for all excitation wavelength sources combined. In each case the white light reflection spectrum can be included or excluded from the PCA analysis. Furthermore a peak-fitting algorithm was added to investigate the PpIX emissions observed.

Algorithm parameters

The parameters are based on the EDOCAL cancer detection algorithm as it was developed by the AMC. This algorithm was extended to provide additional normalization options that can be controlled by a parameter set. These parameters are tabulated below. In addition a PCA analysis module was added to the code and an option to provide EEM matrix output for selected measurements.

Table 3-1 Algorithm control parameters

Parameter	min	typ	max	Notes
Spectrum normalization				
Wavelength range	475		780	minimum and maximum wavelength for analysis (nm)
Smoothing width		3		+/- nm range
White light correction	0	..	3	no (0), yes(1), (2) re-scale for average WL, (3) store average of all WL
Data normalization	1	..	5	(1) per spectr., (2) avg. pwr. per site, (3) avg. pwr per source, (4) pwr. in set, (5) none
les. / non-les. ref. p. source	1	..	4	(0) none, (1) divide, (2) subtract, (3) reserved, all nonlesion (4), acc. non-lesion(5)
data to source ref.	0	..	8	(0) none, (2..8) divide by non_les spectr. of given source
data to average source ref.	0	..	9	(0) none, (2..8) divide by average of all non_les spectr. of given source
Ratio calculations				
ratio window 1	550		570	first window for ratio (nm)
ratio window 2	630		850	second window for ratio (nm)
double ratio sources	2	..	8	sources indexes (0 results in single ratio)
PCA analysis				
no. PCA components		3		components to keep
PCA type	-1	..	1	combined (1) or separate (0), -1 for no PCA
White light in PCA	0	..	1	no (0), yes (1)
EEM				
Measurement index	..	682	..	Index of measurement to provide EEM matrix

The wavelength range is used to select a wavelength range from the spectra for analysis. The spectra are smoothed for noise reduction over a smoothing width (applied in positive and negative direction so the total smoothing width is twice the number provided. The white light correction can be turned off or on (white light correction, when used, is always normalized in the source spectrum) and the whited light correction can be re-scaled for the average of all the white light reflection spectra in the code such that the white light correction only contains the deviation from the average white light reflection spectra resulting in much less spectrum weighting.

Data normalization occurs by integration of the power over the 480-680 nm range and normalizing spectra in that power per individual spectrum. Other options are to normalize in the average power per site (integrated for all sources), to normalize in the average in the average power per source within the set of a patient and to normalize in the total power in a measurement set of a patient. However it was found in practice that the coupling of the probe to tissue in the oral cavity is a source of variation such that the additional normalization options have limited value.

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In the EDOCALD project for each measurement in a lesion, a contra-lateral measurement site is available and options have been added to directly compare lesion measurements to non-lesion measurements. This may help to reduce variation due to differences between patients. Typically a data set includes 3 lesion and 3 non-lesion measurements; the non-lesion spectra can be averaged (per excitation source) and then all spectra can be normalized in these averaged spectra. This can be done as a ratio (divide) or as a subtraction. Alternately normalization can be performed in the average spectrum of all the non-lesion spectra in the entire group of patients and such an average spectrum can be computed and stored. It is also possible to reference all spectra to the average non-lesion fluorescence spectrum of a given source, such that all results are relative to that excitation source for that patient. This can also be applied such that the average fluorescence spectrum of all non-lesion sites for that given source is used as a reference. For ratio calculations the integration windows can be provided for a first and a second sample out of a spectrum and source indices can be selected to compute double ratios.

PCA analysis was added and can be selected to perform PCA analysis of the normalized data per excitation source or PCA analysis for the combined spectra of all excitation sources. In both cases the white light reflection spectra can optionally be included in the PCA analysis. EEM results can be provided for inspection.

Measured spectra for malignant tissue and another non-malignant site are shown below, both show emission peaks at 635 and 705 nm but for the malignant case these are much stronger. Their origin is not certain because such strong peaks are not expected from tissue.

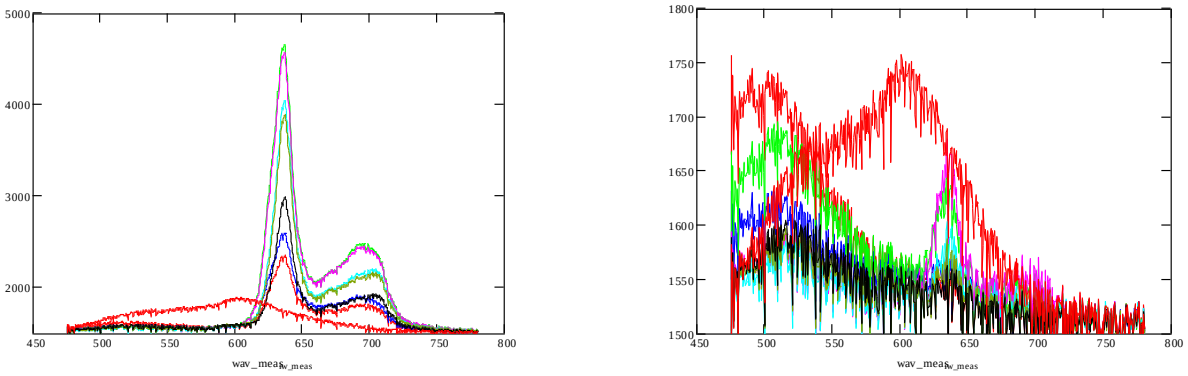

Figure 3-1 Malignant site spectra (left) and non-malignant spectra (right) as measured, Y axis in counts (AU)

The excitation sources are 365, 385, 400, 405, 415, 420, 425 nm respectively, represented as different curves and the white light reflection spectrum is shown in read. There is some noise in the malignant spectra but the overall intensity to noise ratio is good. Note the offset in the spectra of around 1500 counts; this is the dark background of the spectrometer. The non-malignant spectra appear to be very noisy and have relatively low intensity. However the observed “noise” is dominated by the background level of the spectrometer that varies from bin to bin but is relatively consistent over time. A large fraction of the noise is due to pixel offset in the spectrometer, after correction for the dark measurement (where the dark spectrum is averaged over a number of measurements) an acceptable SNR is obtained:

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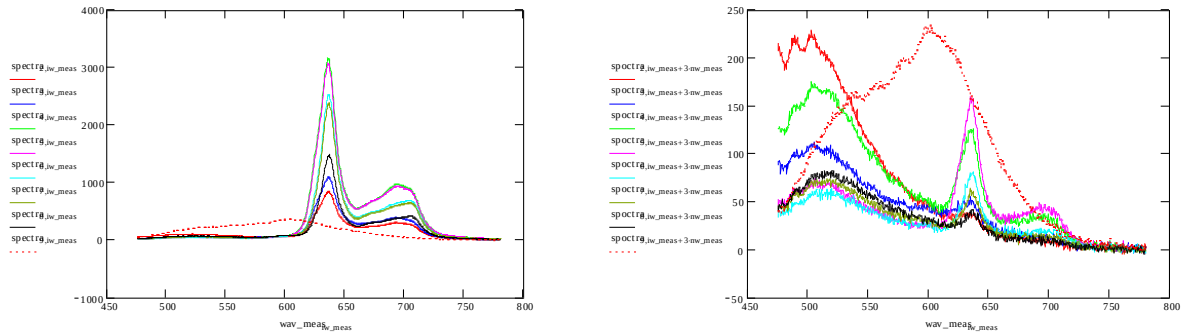

Figure 3-2 Lesion (left) and non-lesion (right) spectra after background correction, Y axis in counts (AU)

Single ratio analysis of data

Application of the single ratio algorithm results in specificity and sensitivity as shown in the next figure where sensitivity and specificity are defined as:

- Sensitivity: percentage of sick people correctly identified as having the condition
  - Healthy people in-correctly identified with the condition do not count
- Specificity: percentage of healthy people correctly identified as not having the condition
  - Healthy people in-correctly identified with the condition are lost from the count, but if population identified with the condition is small then a few false detections do not affect specificity

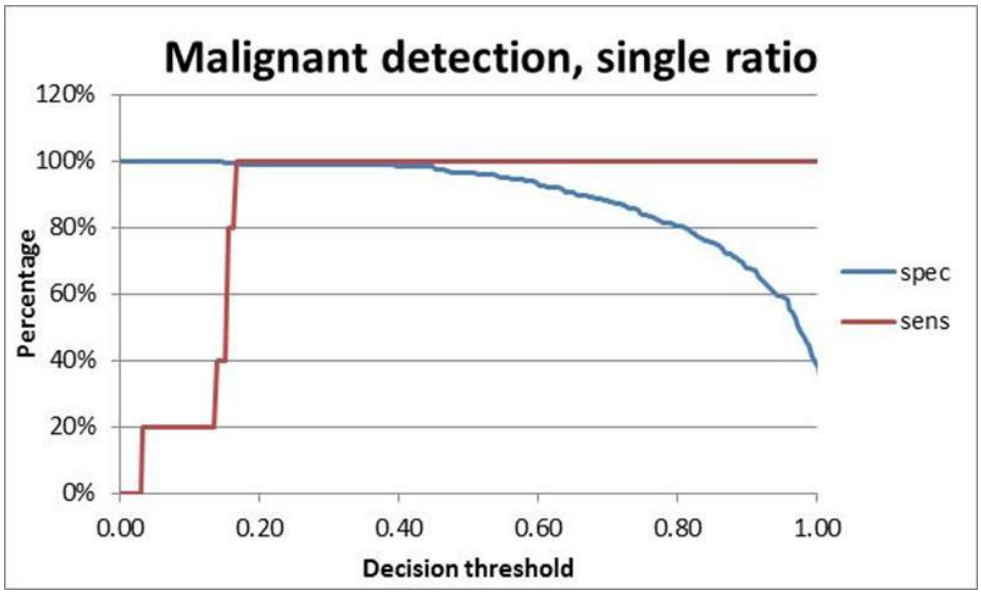

Figure 3-3 Sensitivity and Specificity for detection of malignant tissue as a function of detection threshold for single ratio with 420 nm excitation source

Both sensitivity and specificity for malignant tissue detection exceed 98% for a detection threshold in the 0.2-0.4 range. However there are only 5 malignant tissue measurements available from 2 patients in the set.

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Sensitivity and specificity crossover

In order to find an optimum detection algorithm the cross-over point between sensitivity and specificity as shown in Figure 3-3 is automatically detected. This results in the best available sensitivity/specificity compromise available for the detection method. This was done for all the available excitation wavelengths and for the white light source.

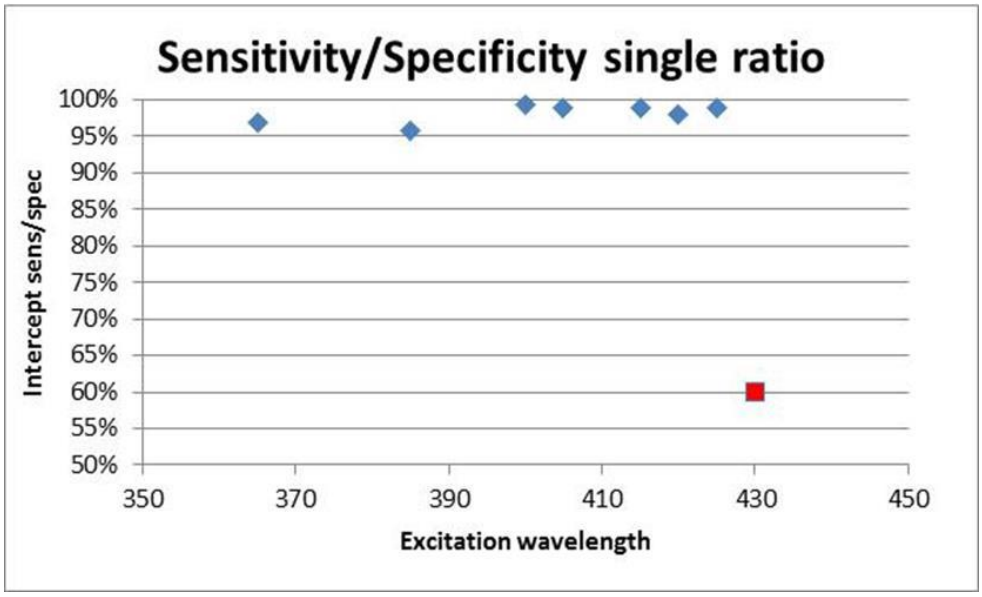

Figure 3-4 Sensitivity/specificity available with single ratio for detection of malignant tissue

The result shows that each of the excitation wavelengths results in a good sensitivity and specificity for malignant tissue detection in the given data. This result is obtained whether white light correction is used or not. The white light measurement alone cannot be used. For potentially malignant sites the sensitivity is considerably lower as shown below.

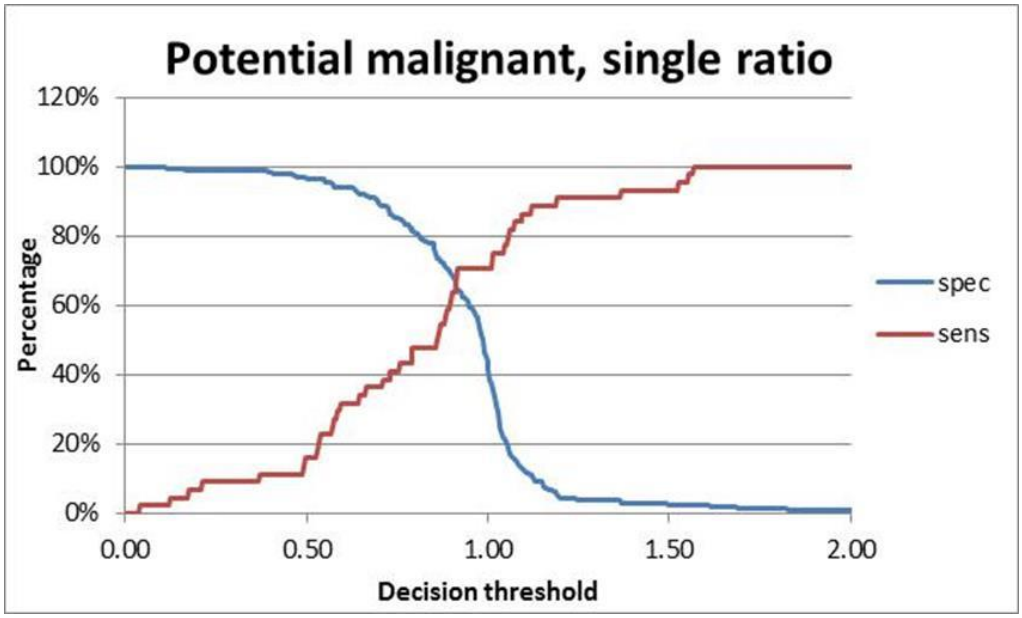

Figure 3-5 Single ratio specificity and sensitivity for potentially malignant sites

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For the different excitation wavelengths the potentially malignant tissue detection is between 60 and 70% sensitivity/specificity for each of the sources. This is illustrated in the figure below (bio 4 is malignant, 3 is potentially malignant, 2 is inflammatory and 1 is benign).

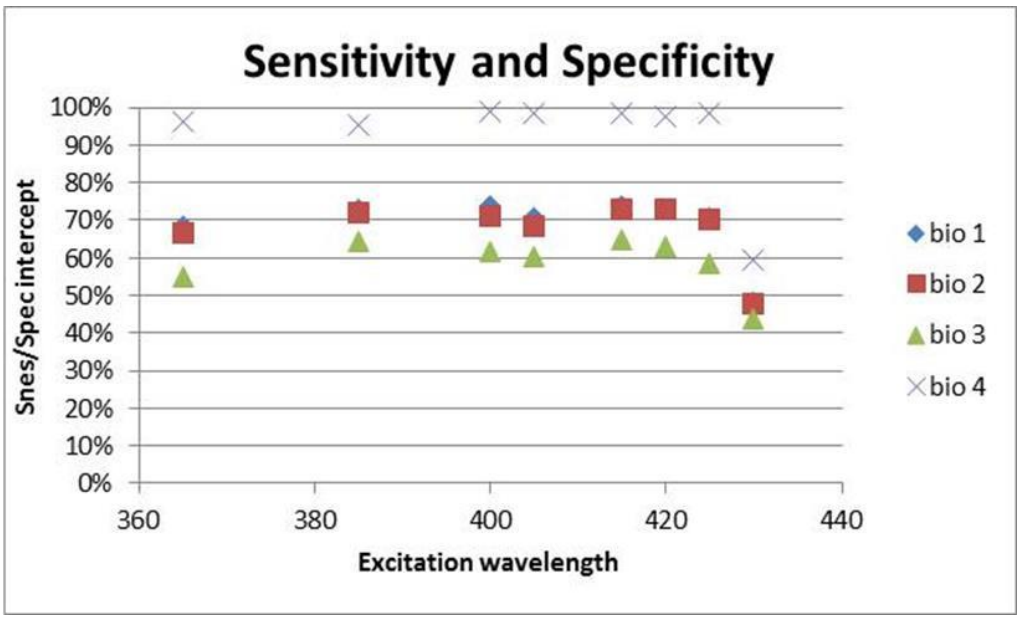

Figure 3-6 Sensitivity and specificity for different biopsy grades for different excitation wavelengths

Clearly the detection of potentially malignant tissue is difficult; up to 2/3rd or less of such tissues are expected to eventually develop into malignant lesions, but it is unknown which biopsies would, so that a very high detection sensitivity/specificity may not be expected.

Double ratio analysis of data

The single ratios are computed for each excitation wavelength. For each combination of excitation wavelengths a double ratio can be computed and for each of the resulting double ratios the best sensitivity/specificity compromise was determined. This did not result in a further improvement of results. The best results are in the 60-80% range for malignant tissue, in this case less than for single ratio detection. This may be understood in light of the fact that malignant tissue detection was based on the detection of PpIX emission, that results in a very strong single ratio signal and much weaker double ratio signal.

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PCA analysis

Principal component analysis was performed on the entire dataset, in this example with normalization of the data in average non-lesion spectra. The first 3 principal component 'eigen-vector' spectra are shown below:

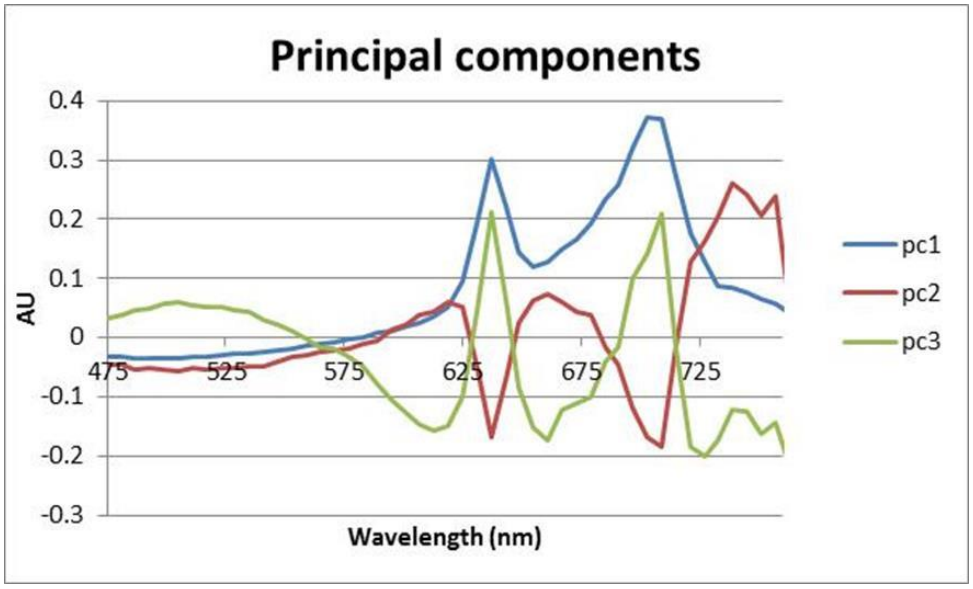

Figure 3-7 First 3 principal components

The components are normalized to the same scale for the plot, most variation is in pc1. The first principal component pc1 has 10x the variation of pc2 and pc3. The signals are dominated by PpIX peaks. Note it was verified that the components are mutually uncorrelated, as should be expected for orthogonal eigenvectors, even though they appear similar. The first two principal components for 400 nm excitation are shown below.

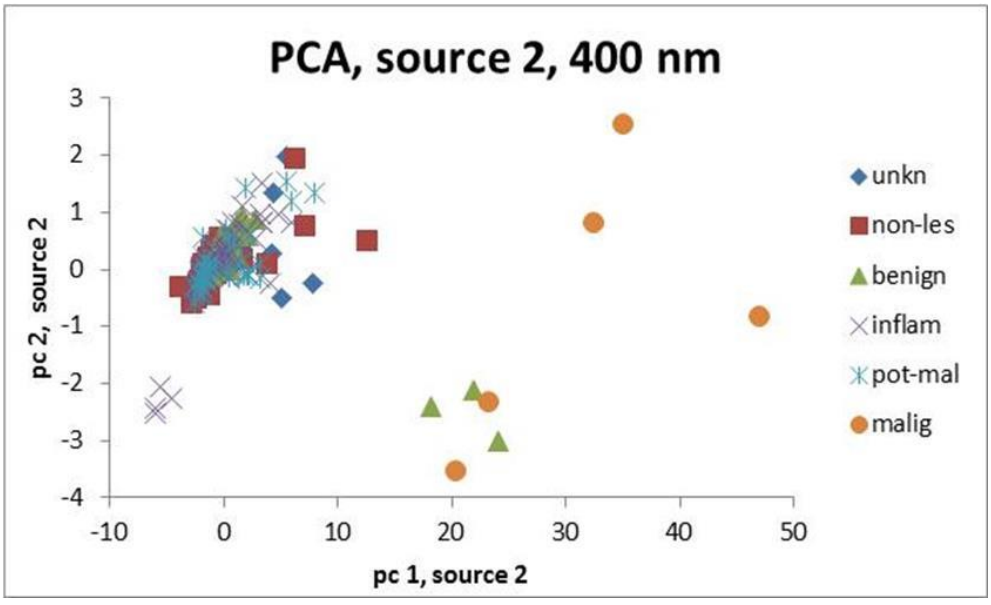

Figure 3-8 Principal component analysis 400 nm source

The PCA analysis results in clear separation of the malignant cases from the rest of the measurements, only a few benign samples mix with the malignant samples. However, as

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with the single or double ratio analysis these results are dominated by the PpIX emission. By combining PCA results from multiple excitation wavelengths the benign samples can be separated from the malignant samples as shown in the figure below.

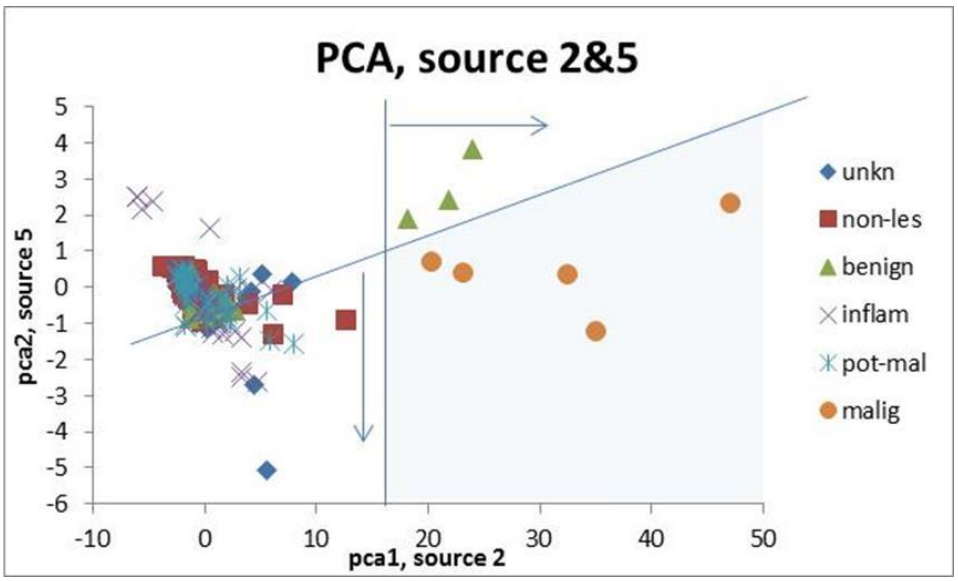

Figure 3-9 First principal component of 400 nm and second principal component of 420 nm source combined

The combination of principal components of the two excitation sources results in a better separation of the malignant tissue samples from the few benign samples that mixed with the malignant sample results and in permits unique detection of malignant tissue. Results to the left of the vertical line and below the tilted line (shaded area) can be identified as malignant tissue. The detection of potentially malignant tissue is equally difficult in the PCA analysis as it is in the (double) ratio analysis. As shown below the tissue spectra of potentially malignant sites are not clearly separated from the other tissue spectra.

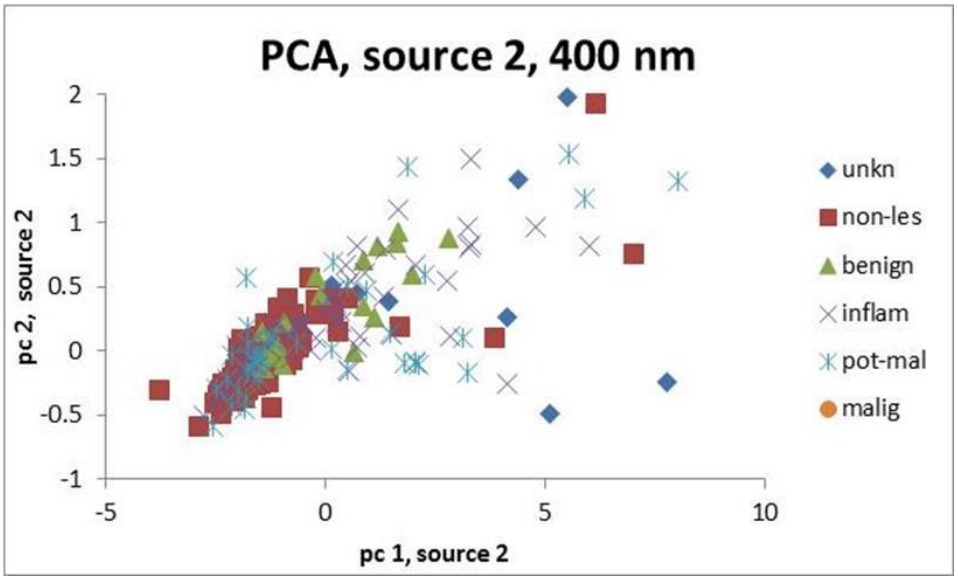

Figure 3-10 PCA analysis, zoomed into samples that are not clearly malignant

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Spectrum fitting

A spectrum fitting algorithm was used to fit individual components to spectra where each component is a modified Gaussian or Lorentzian. For each component the peak wavelength, peak width and drop-off of the tails are set. Then the amplitude of each individual component is fit to the measured data. For the first peak at around 500 nm the peak width and peak wavelength are optimized in the fit. Examples of spectrum decomposition are shown below for malignant lesion and non-lesion samples.

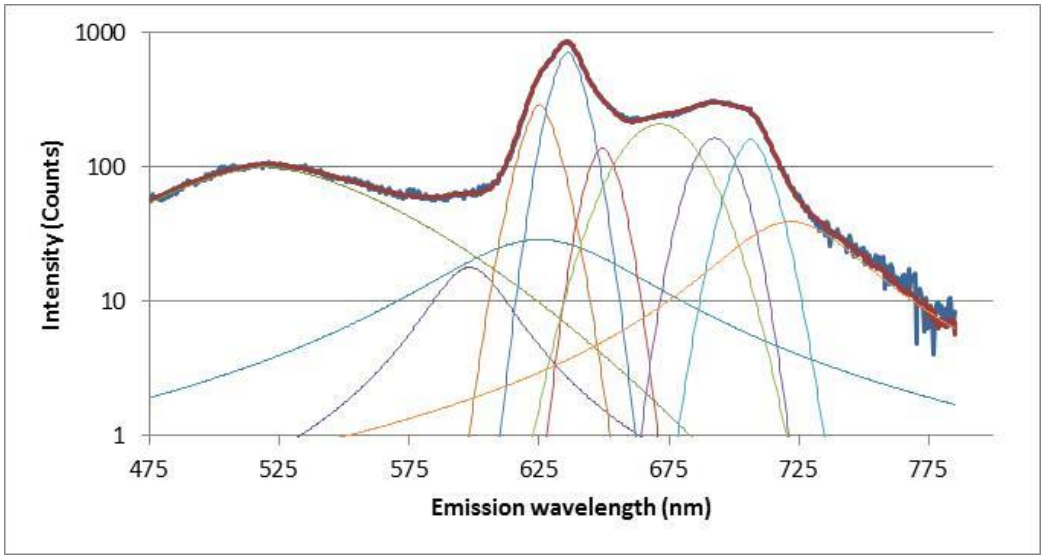

Figure 3-11 Spectrum decomposed into individual components (malignant)

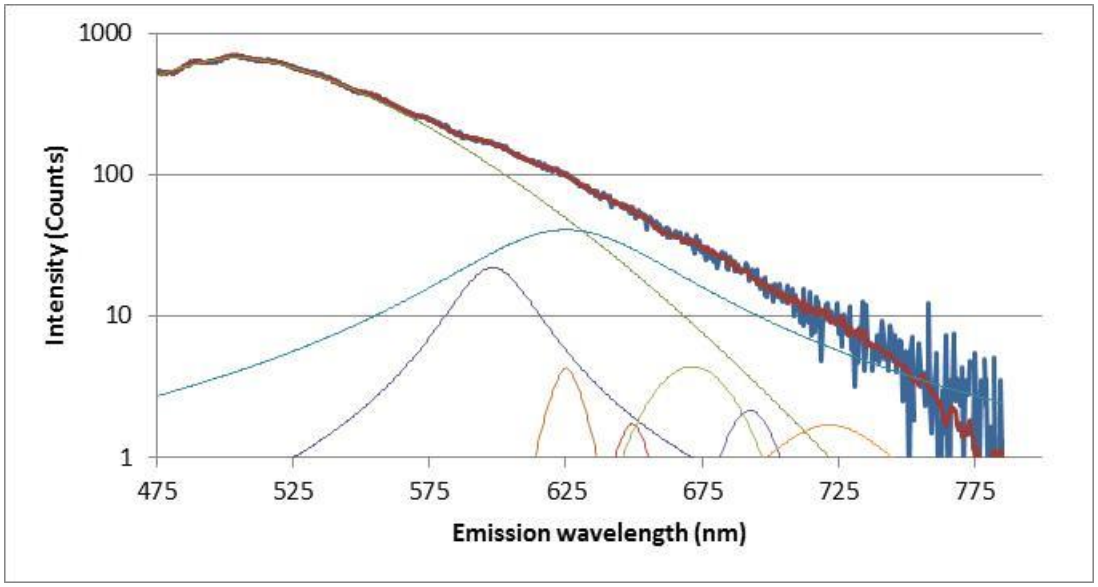

Figure 3-12 Spectrum decomposed into individual components (non-lesion)

The sum of the fit spectra (red) is plotted on top of the measured data (blue) and the individual components in the fit (except ripple correction spectrometer, quite visible in the 500 nm range) are shown as thin lines. As shown in these examples the residual error after fitting is low. Thermal noise from the spectrometer in the measured curves is visible for low intensities and corresponds to about 2.5 counts rms. Note these fits are performed to data that has not been smoothed. Fits were performed to all the 2200 measured spectra.

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In order to inspect if the fit algorithm is good enough to describe the measured spectra the residual error of all of these fits, normalized in the average power in the spectra is shown in the next figure.

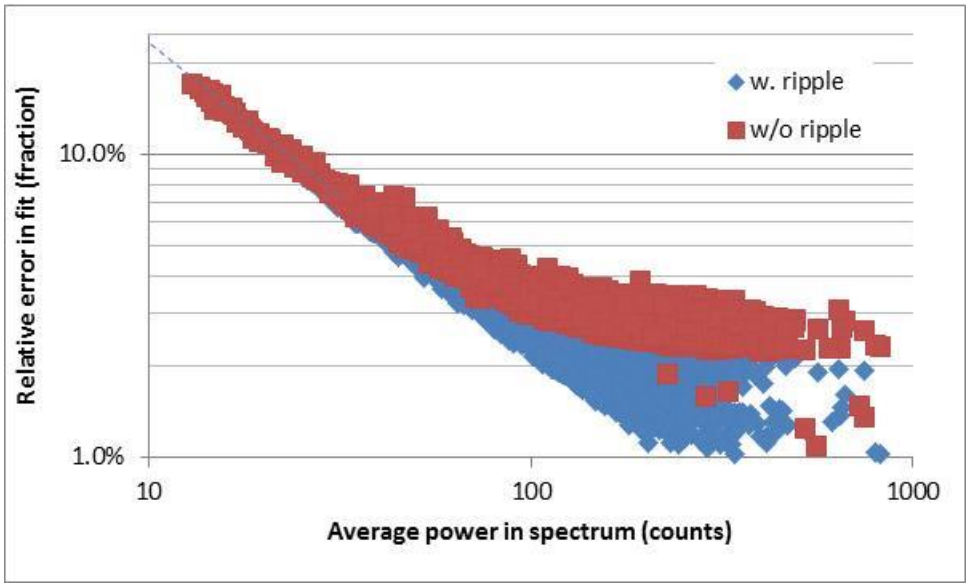

Figure 3-13 Relative fit error for all spectra

For large enough power in the measured spectra the fit error drops to ~2.5%. The spectrum analyser in the EDOCALD demonstrator and prototype can have some residual ripple that is not captured in the spectral components fit to the data. Permitting the fit routine to add this ripple component (a fixed function for all spectra) further reduces the fit error to about 1.5 % (so that 98% of the spectral content is described by the components fit to the spectra). For weak spectra the fit error is higher due to the thermal noise in the measured spectra that has an rms value of close to 2.5 counts (25% at an average spectrum power of 10, top of the scale in the plot). So it can be concluded that the set of emission peaks used to fit the spectra is sufficient to describe all measured features in the spectra. The set of peaks used to fit the spectra is tabulated below.

Table 3-2 Set of peaks fitted to fluorescence spectra

Type	center wavelength (nm)	peak width (nm)	power (nm)
Gauss	506.8	70.9	1.5
Lorentz	598.3	16.0	2.0
Lorentz	625.3	40.2	2.0
Gauss	625.2	9.0	1.6
Gauss	636.0	8.3	1.6
Gauss	649.4	8.7	1.8
Gauss	671.5	21.2	2.0
Gauss	692.7	12.5	2.0
Gauss	706.5	10.6	1.7
Lorentz	721.8	27.7	2.0

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All the parameters are fixed in the fitting procedure except the centre wavelength and width of the first peak at 506 nm. This peak shifts with excitation wavelength. The fitting procedure optimizes these two parameters and the individual amplitudes of the components.

The primary purpose of the spectral fitting procedure is to separate the strong PpIX fluorescence from other fluorescence such that spectra could be corrected for the PpIX fluorescence. It was found that several peaks in these spectra are strongly correlated.

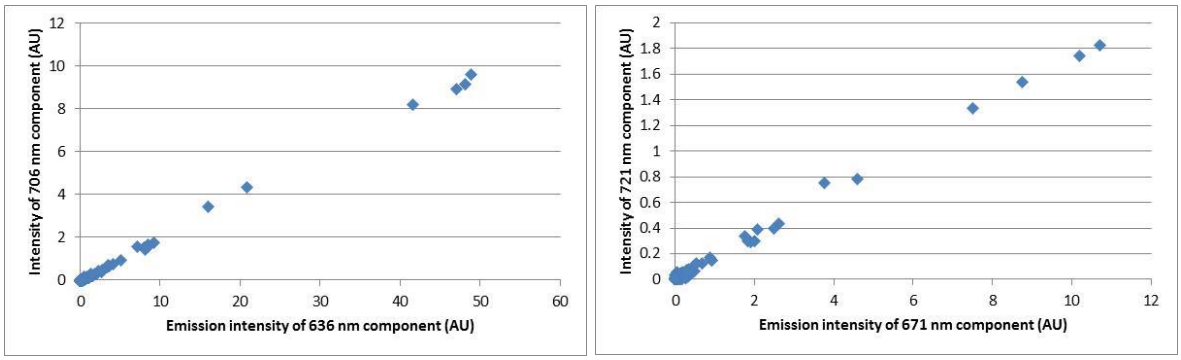

Figure 3-14 Correlation between 636 and 706 nm emission (left) and between 671 and 721 nm emission (right)

The 636 and 706 nm emission peaks are both due to the same PpIX emission (left). Peaks at 625, 649, 671, 692 and 721 nm are also closely correlated (right example of 671 and 721 nm). These peaks are also closely related to the PpIX emission but not identical as shown below for 636 and 692 nm (left), apart from a few exceptional data points (3 points from a malignant site measurement) all these peaks describe the same information and this implies that most of the spectral information in the wavelength range beyond 625 nm is completely dominated by PpIX or related fluorescence.

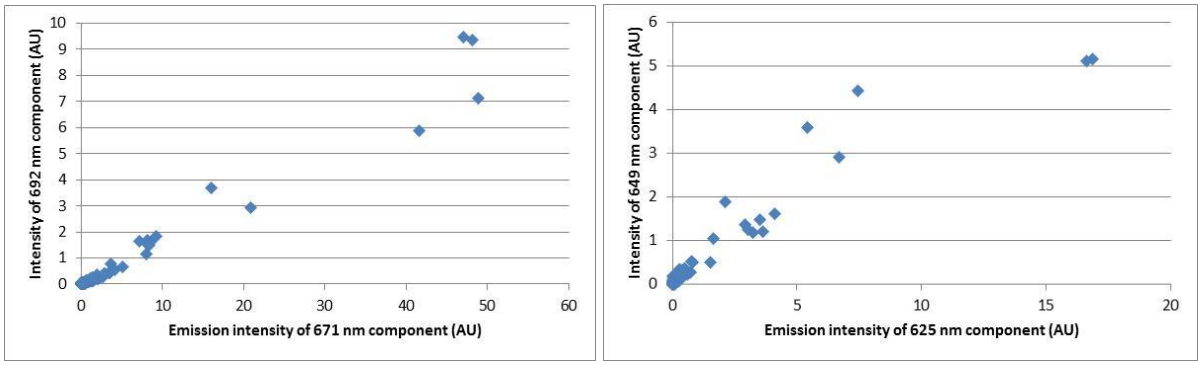

Figure 3-15 Correlation between 761 and 692 nm emission (left) and between 625 and 649 nm emission (right)

In case this fluorescence is indeed strongly related to malignancy, as was observed in this limited set of malignant data that was available, then it is effective for detection. It dominated the PCA analysis and the (double) ratio calculations as well and also the normalizations in spectrum power due to the wavelength ranges selected. However in case it is dominated by other factors, such as bacteria present in the mouth, then this implies that it will obscure any fluorescence information in the wavelength range above 625 nm such that the EDOCAL algorithms cannot be applied. Note that this could be a fundamental difference between

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measurements in the oral cavity from measurements on other tissue complicating reliable fluorescence measurements in the oral cavity.

There are a few peaks that were needed to fit the short wavelength range. These are located at nominally 505 and 598 nm and can be used in case the PpIX related information must be discarded. The location of the 505 nm peak and width are excitation wavelength dependent such that ~4 parameters (2 for peak amplitude) are needed and sufficient to describe in the information in the short wavelength range. For each excitation wavelength these parameters are available. To circumvent the problem of varying PpIX emission the spectra can be normalized amplitude in the first wide fluorescence peak around 505 nm such that for normalized spectra 3 parameters may remain per excitation wavelength.

Note that for malignant tissue (biopsy grade 4) the intensity at the main 505 nm peak is generally lower than for other biopsy grades as may be expected. However for potentially malignant tissue (biopsy grade 3) this is not observed so it does not help to improve detection. Also, it has been observed that the coupling of the probe to tissue in the oral cavity is not as reproducible as in a lab environment such that use of this absolute fluorescence intensity without normalization as a decision criterion is not preferred.

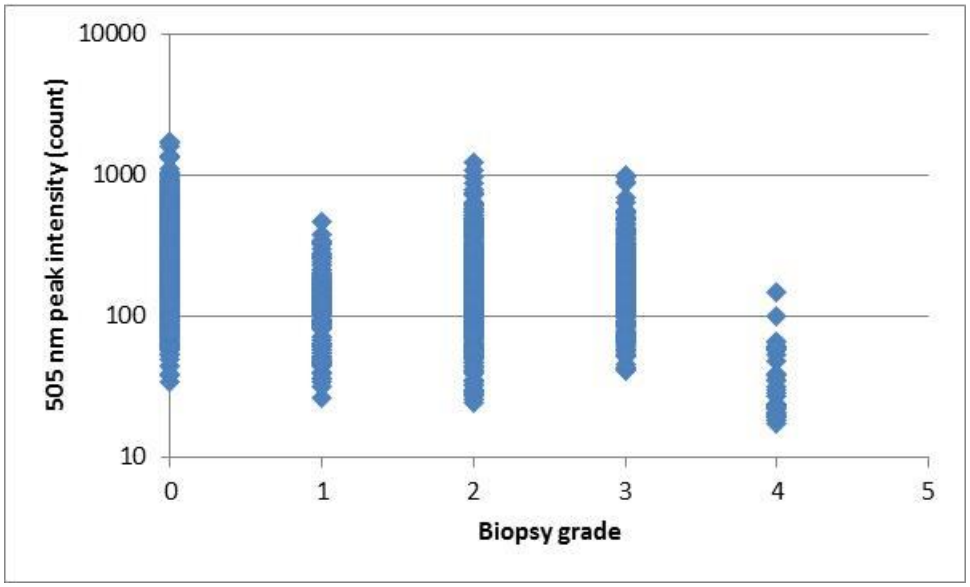

Figure 3-16 Absolute intensity of 505 nm emission peak for different biopsy grades

Initial analysis of the short wavelength range only did result in a systematic peak wavelength shift of the 506 nm peak with excitation wavelength. However, thus far it has not yielded improved detection of potentially malignant tissue.

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Conclusion

It is concluded that using a single ratio calculation as used in the EDOCAL project, malignant tissue could be detected with 98 % specificity and sensitivity. Double ratio calculations as used to improve detection in the EDOCAL project do not result in a further improvement. With PCA analysis the sensitivity and specificity could be improved to 100%. However the data set of malignant tissue is very small (5 sets of spectra collected from 2 patients out of a group of 48 patients) so that more data is needed to gain confidence. It was further shown, using PCA, EEM and spectrum peak fitting that the detection is primarily based on the detection of strong PpIX fluorescence. It is not obvious whether this is tissue related or due to other factors such as bacteria in the oral cavity. Further spectrum peak fitting analysis has shown that all measured spectral features at wavelengths of 625 nm and beyond are strongly correlated to the PpIX fluorescence. In case further research shows that the PpIX fluorescence is due to other factors not related to malignancy then this implies that spectra cannot be used for wavelengths beyond 625 nm because those signals are dominated by such other factors that are present in the oral cavity. If so spectrum analysis must be limited to the 475-625 nm range and further research is needed to develop a new spectral analysis method because both single and double ratio methods developed in the EDOCAL project can no longer be applied.

Detection of potentially malignant lesions is difficult; specificity and sensitivity in the range of 60-70% were obtained. This may be understood within the context of other work that shows that only a fraction of such lesions develop into malignant lesions. Further research is needed with a finer scale for biopsy results to determine if a better correlation can be established.

References

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[2] Photochemistry and Photobiology, 2000, 72(1): 103–113

[3] Appraisal of Screening for Oral Cancer, A draft report for the UK National Screening Committee

[4] Oral Oncology 51 (2015) 883–887

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3.3 WP5 Clinical Studies

3.3.1 Description of Work package 5

In WP5 the test system will be applied to patients scheduled for tissue biopsies. The system results are then compared with conventional histopathology so that the technology can be validated as clinically relevant and accurate. Reproducibility and inter observer variation will also be assessed and other factors related to ease of use and time taken for the test. From this, an assessment of commercialisation will be possible.

Studies at the Lund Institute of Technology have demonstrated the fluorescence spectrum measured from a fluorophore in tissue is affected by the absorption and scattering properties of the tissue, as well as by the measurement geometry. Analysis was made of this effect with Monte Carlo simulations and by measurements on phantoms. The spectral changes can be used to estimate the depth of a fluorescent lesion embedded in the tissue by measurement of the fluorescence signal in different wavelength bands. By taking the ratio between the signals at two wavelengths, it has been shown that it is possible to determine the depth of the lesion. Simulations were performed and validated by measurements on a phantom in the wavelength range 815-930 nm. The depth of a fluorescing layer could be determined with 0.6-mm accuracy down to at least a depth of 10 mm. Monte Carlo simulations were also performed for different tissue types of various composition.

Preliminary governance and preparation for the Clinical studies
The following is a summary of progress towards achieving ethical permission to proceed with the use of the first generation Demonstrator (EDOCAL-DEMO) of a real-time Photonic Cancer Detector (PCD) in the clinical setting that will initially be used for early diagnosis of oral cancers.

The list of tasks below was preceded by preparation of extensive documentation describing the clinical application, the implications for patients who are eligible to participate in the study, and the requirements in terms of personnel and procedures for successful delivery of the EDOCALD project in the clinical setting:

1. Obtained insurance & indemnity, and subsequently sponsorship from UoD for the EDOCALD study to be conducted in clinical settings

2. Integrated Research Application System (IRAS) procedure completed with protocol and tailored Patient Information Sheet & Consent form for EDOCALD

3. NHS R&D application was submitted and approval received, along with the site specific investigations (SSI) application & approval for both Dental School and Ninewells Hospital

4. EDOCALD was confirmed as a trial that did not involve an Investigational Medicinal Product (IMP) as defined by the MHRA, and therefore do not fall within the scope of the Medicines for Human Use (Clinical Trials) Regulations 2004.

5. The fact that EDOCALD was deemed as a non-invasive, non-CTIMP project led to the invitation to apply for proportionate review, which was submitted on 5th March 2014.

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6. The final REC review was held 21st March 2014 – Professor Peter Mossey attended in person to present case and answer queries, and we were notified on 28th March 2014 that the ethical permission had been granted.

7. Dr Michaelina Macluskey Consultant Oran and Maxilliofacial surgeon, Dr Elizabeth Theaker, Consultant in Oral Medicine and Professor Peter Mossey participated in discussions surrounding standardisation of the methods to be used in the photographic capture using the first generation Demonstrator (EDOCAL-DEMO) Photonic Cancer Detector.

8. In preparation for the commencement of the clinical part of the EDOCALD project, the job description for EDOCALD research nurse was sent to the human resources (HR) department of the University of Dundee for approval & advertising.

To estimate the sample size for the study prior to recruitment, a power calculation was carried out. It was estimated that to detect a **20 %** (x = 0.2) difference in the detection of oral cancers using auto fluorescence (Rana et al, 2011) with a significance level of α = 0.05 (a = 1.96) and a power of 90 % (β = 0.1; b = 1.28), a **sample size of 51** was calculated; Using the same equation, a **10 %** (x = 0.1) difference in the detection of oral cancers with a significance level of α = 0.05 (a = 1.96) and a power of 90 % (β = 0.1; b = 1.28) would require a **sample size of 202.** It was agreed that in the interests of feasibility, and with the difficulties encountered with the demonstrator equipment, this EDOCALD study would aim to recruit a sample size that will detect a 20% difference.

The collective result of the above procedures was that ethical permission was granted on schedule for the EDOCALD study to be conducted on two sites in Dundee; Dundee University Dental School and Ninewells Hospital; and for a research nurse to be recruited to facilitate the clinical trial after the demonstrator has been delivered and tested.

Assessment of the Clinical Validity / safety of the system

The demonstrators were delivered on the 18th of November 2014. The team, consisting of Elizabeth Theaker, Michaelina Macluskey, Fiona Ord and Jill Gouick, spent three sessions familiarising themselves with the demonstrators (24/11/14, 8/12/14, 15/12/14) before they went for risk assessment.

When the demonstrators returned four sessions (5/1/15, 12/1/15, 19/1, 26/1/15) were used to look at inter examiner variation and intra-oral site variation in normal volunteers. Two volunteers were involved volunteer 1 had 3 readings per site at 10 sites by 1 operator. Volunteer 2 had 3 readings per site at 8 sites by 2 operators. During this time some software problems developed so demonstrator 1b was sent back to Holland for trouble shooting. We continued with demonstrator 1a but this also developed problems.

Meanwhile there was close collaboration with Professor Harry Moseley, Dr Julie Woods and Dr Ronan Valentine at the Photobiology Unit at Ninewells Hospital who had extensive prior experience with EDOCAL and EDOCALD and fluorescence diagnosis were providing the expertise to test the lasers clinically. They were also responsible for the general safety and risk assessments and addressing issues of infection control (through the design and supply of sterile sheaths for the intra-oral component of the EDOCALD demonstrator probe).

Patient recruitment

Patient recruitment commenced as soon as the indemnity was granted on the 20th of April 2015. Letters of invitation were sent out to all new biopsy cases attending Dr Theaker and Dr

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Macluskey's clinics at Dundee Dental Hospital and Ninewells Hospital. In June 2015, an amendment to the ethics application was sought to allow the recruitment of patients on the day and this was granted by EoSRES. The patients were provided with an information leaflet and once they agreed to be involved a consent form was signed which was photocopied so that one copy went into the patient's notes, one was given to the patient and one was kept in file by the research nurses. Patients were given a unique number which was used as an identifier for the lesion database and for the demonstrator programme.

Protocol for recruiting participants

1. Patients identified as meeting inclusion criteria
2. Patient invitation sheet posted (if appointment booked in advance)
3. Oral mucosa viewed to determine if lesion appropriate for inclusion on the day
4. Informed consent taken
5. New patient ID number is added to system

(EDT clinic 4 numbers beginning with 10 , MM clinic 4 numbers beginning with 20)

Clinical visit and capture of the pathology using laser and light

Protocol for capturing measurements:

1. Measurements taken on three standards in a darkened room.
2. New patient ID number is added to system (ET clinic 4 numbers beginning with 10, MM clinic 4 numbers beginning with 20).
3. Confirm site with operator and select site on menu.
4. White light image is taken.
5. Lesion mapped on white light image using 3 points.
6. Blue light image taken – replicating white light image.
7. Indicate on image where lesion is situated for light source readings to be measured.
8. Lights turned off and record light source measurement on the intended biopsy site (3 readings), lifting the probe between each reading using a disposable sheath for cross infection control.
9. Take light source measurements on opposing normal side of mouth (3 readings), lifting the probe between each reading.
10. In cases with multiple lesions they had three measurements at each site.
11. Measurements taken on three standards in a darkened room at the end of the session.
12. All measurements backed up on the laptop.

From June 2015, the one functional piece of equipment (demonstrator 1b) was transported between the two hospital sites by the research nurses.

Handling of the biopsies via Pathology at Ninewells

Once the readings had been taken the biopsy was carried out which involved injecting local anaesthetic into the area, sampling a representative area of the lesion and placing the tissue directly into formal saline and suturing the wound. The biopsy sample was labelled and the pathology form completed. Once all the samples had been collected these were sent to the Pathology department at Ninewell's hospital. The report was generally available three

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weeks later and the patients were reviewed then to tell then the result of the biopsy. At this point the details of the pathology were entered into the database by the research nurses.

Various tissue structures were evaluated as part of this study, including: keratin cell layer, epithelial layer, basement membrane, lamina propria and other microanatomical structures. Two independent assessors (clinician and pathologist) assess the images and will be asked to comment on the cellular structures and changes involving the tissue structures in non-blind fashion. The laser and imaging data was accessed remotely by the team in the Netherlands for analysis at arranged times. They were able to access the images and the measurements. Periodically the lesion database was also sent to our colleagues in the Netherlands so that the measurements could be matched to the pathology.

User Validation and data security

Generally the patients seemed happy to be involved although the uptake was approximately 60% and it was difficult to recruit patients with cancer as they generally were seen by the maxillofacial consultants on their clinics. The patients did not report any discomfort associated with the probe. The demonstrator was easy to use after a period of training. It does take some time to set up and calibrate the machine before using it on clinic so it is essential that it is set up in advance. The packaging is a little bulky and we had concerns that the probe was not damaged so is transported in a large flat box. Once set up the camera system is easy to use and the examination and measurement can be carried out in a timely fashion certainly within 10 minutes. The probe can access all parts of the oral cavity. We initially used the foot switch to take pictures and measurements but we no longer do this relying on one of the research nurses to operate the software.

The recorded intra-oral photograph(s) will be stored under a unique identifier number in a linked database. The study system will be based on the protocol and CRF for the study and individual requirements of the investigators. UNIVDUN has a data security management system in place. The guidelines as defined in ftp://ftp.cordis.europa.eu/pub/fp7/docs/privacy.doc will be followed regarding data collection, preservation, protection and security. Biological samples will be collected and preserved, as per usual NHS practice which including details of the duration of the collection and preservation.

Generation of a Pathological Database

A password protected spreadsheet was populated with the unique identifier for each patient, their CHI (hospital number), gender, the site of the biopsy/ lesion and the category of the pathology. It was decided that rather than using the actual pathology details the samples would be categorised as 1 normal, 2 benign inflammatory, 3 potentially malignant and 4 malignant. For category 3 these were classified as mild, moderate or severe dysplasia.

Contribution to WP8: Exhibit Demonstrator at Medical Conferences

Dr Theaker and Dr Macluskey were asked to speak at a National Training Event for Core Dental Trainees held in Dundee on the 29th of October on Oral Medicine. This allowed them to show these dentists how the demonstrator is used and give an outline of the study thus far. This was well received.

An abstract has been accepted for SPIE Photonic West conference which will take place in San Francisco I February 2016. The title of this presentation is “Clinical validation of a multi-

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wavelength (u.v. to visible) laser system for early detection of oral cancer" and will be presented by Dr Macluskey.

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3.4 WP6 Functional Validation

3.4.1 Objectives

WP6 had 3 objectives:

1. Create test conditions, review and release functional specifications for EDOCAL Demonstrator
2. Revise test conditions and release functional specifications as result of feedback from evaluation of test systems
3. Test final EDOCAL Demonstrator against functional specifications

3.4.2 Deliverables

WP6 produced the required 6 deliverables:

1. Draft test conditions
2. Released test conditions
3. Test setup functional validation
4. Functional validation
5. Functional validation released

During the validation process the characteristics of all parts of the system were investigated and tested against the specifications laid down in the User Requirements Specifications, including:

- Spectral properties of light sources,
- Spectral properties of the light path from light source to tissue sample,
- Spectral properties from illuminated tissue sample to detector,
- Optical power levels at device output,
- Optical power levels at the probe tip
- Exposure times
- Noise levels
- Electrical Safety
- Environmental (temperature and EMC)

The validation process has shown that the systems (Demonstrator 1A, 1B, 1C and the LEDs versus LASERs system) produced during the project produced reliable and repeatable measurements where the wavelengths, optical powers and exposure times were consistent with the specifications laid down in the User Requirements Specification. Analysis of the spectra produced by both the laboratory measurements at the Photobiology unit at the University of Dundee and the clinical measurements at the Ninewells site and at the School of Dentistry have also shown this consistency.

3.4.3 Learnings from the validation process

During the evaluation of the test setup systems a number of issues appeared, the solutions for these issues lead to modifications in both the test setup systems and the validation documents.

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3.4.3.1 Need for Long Pass Filter

The first issue to be identified was the appearance of numerous unexpected peaks in the measured Demonstrator 1A spectra from both normal healthy tissue and from reference compounds. Analysis of these peaks and calculation of the wavelengths lead to the belief that they were due to higher order diffraction components of the excitation light sources in the spectrometer diffraction grating. The introduction of a fibre-coupled optical long pass filter with a cut-off wavelength of 450nm in the optical signal path to the spectrometer suppressed the intensity of the excitation light to a level such that the higher order diffraction components were no longer a hindrance in the measured fluorescence spectra. This system modification was also incorporated into the subsequent Demonstrator 1B and Demonstrator 1C optical path designs.

3.4.3.2 Need for clean handling of Optical Fibers

The second issue to be identified by the evaluation of Demonstrator 1A was that the optical power output at the tip of the bifurcated probe was significantly less than expected for the 430nm LASER source. Careful examination showed that a fiber tip on one of the legs of the laser optical fiber combiner was contaminated with a finger print. Cleaning with isopropyl alcohol did not bring the optical power to the level required. To solve the problem for Demonstrator 1A the white (halogen) light leg of the combiner was swapped with the 430nm laser leg. For the subsequent systems the procedures were modified with regards to handling the optical fibers during assembly of the system, powder free latex gloves were used.

3.4.3.3 Need for EMC robustness

During the initial practice sessions after the delivery of Demonstrator 1B to Dundee School of Dentistry, the systems began to display erratic operation such as the laptop computer suddenly loosing communication with the EDOCALD system, capture sequences been made where no light was observed at the bifurcated probe tip. These problems were replicable in normal use at the 2M development site. The problems were traced down to EMC issues, the Dundee Ninewells and School of Dentistry sites were significantly more EM polluted than the development site at 2M. After taking appropriate actions to remedy the situation, such as system grounding, shielding and EMC filtering, the issues were solved and clinical measurements could continue. These same solutions were subsequently applied to all systems created during the project.

3.4.4 Conclusion

It is concluded that the delivered systems were conform with the specifications as set down in the User Requirements Specifications, and that after actions were taken to remedy the initial issues experienced in clinical and laboratory sites in Dundee, the systems proved to operate in a reliable and consistent way, providing clinical measurements for use in WP4, the development of data processing and algorithms for the early detection of cancer.

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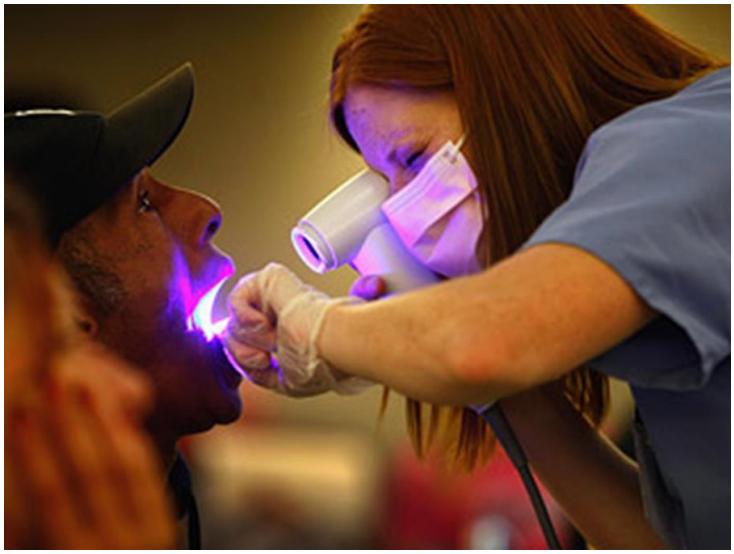
3.5 WP7 Marketing Strategy

Our ***Market Research*** focused on oral lesions has helped us uncover important diagnostic shortfalls among dentists, oral surgeons, emergency care physicians and general practitioners which has in turn helped us to develop a marketing strategy.

The first and most important is the increasing rate of oral cancer, its high mortality rate and **difficulty in achieving early diagnosis**. Oral cancer had 372,000 new cases and was responsible for over 150,000 deaths. The 5 year mortality is approx. 50%. It is the 8th most common cancer for men. Often no visible lesions or discolorations are apparent in the early stage (local). Risk factors include smoking, alcohol and HPV 16. And one is 20 times more likely to develop a second cancer.



HOWEVER early diagnosis results in a more than 80% survival rate.

New research in the UK has shown that one quarter of cancer patients are losing faith in the NHS because they are forced to visit their GP at least three times before they are referred for diagnostic tests. Cancer Research UK scientists from University College London and the University of Cambridge say that many cancer patients are dissatisfied by their care and are losing confidence in doctors and nurses who go on to treat them.

Researchers studied data from 70,000 patients and found that out of the 60,000 people who were diagnosed through their GP, nearly 13,300 had been seen three or more times before they were referred for cancer tests.

Study author Dr Georgios Lyratzopoulos from UCL stated, “When they occur, diagnostic delays are largely due to cancer symptoms being extremely hard to distinguish from other diseases, combined with a lack of accurate and easy-to-use tests. New diagnostic tools to help doctors decide which patients need referring are vital to improve the care experience for even more cancer patients.”

According to the National Institute for Health and Care Excellence “too many GPs are “guessing” whether symptoms could mean cancer, with late diagnosis responsible for the deaths of up to 10,000 people in the UK each year.”

The second shortfall and one highlighted by a survey in Scotland reported several perceived barriers to oral cancer screening. Only 19% of General Dental Practitioners (GDP) reported routinely enquired into smoking habits and 49% did so 'occasionally'. Three percent routinely enquired about alcohol intake, but 68% rarely or never did so. The reasons cited for doing so indicated that they were not comfortable about enquiring about alcohol use. With regards to a thorough soft tissue examination, 41% indicated that a lack of training was an important

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barrier. Forty-three percent cited time factors as a deterrents. Another 31% of respondents also viewed the potential to generate patient anxiety as another barrier.

Initial mismanagement was another reason for referral delay since as many as 74% of patients in one study were given treatment not related to their malignancy, for example mouthwash, antibiotics, analgesics or tooth or denture adjustment. The appropriateness or accuracy of the referral was cited as another reason for delay as in some cases patients were referred to the wrong specialist. Another study that analyzed data from two large national surveys showed that those at the greatest risk of oral cancer had a low likelihood of regularly attending for dental check-ups. Therefore those who do attend and undergo screening are at low risk of oral cancer – there is an 'inverse screening law' present. This presents another problem as clinicians will have a low index of clinical suspicion for patients who are deemed to be low risk. This was highlighted by Yu *et al*., who found that female patients and non-smokers had longer delay periods as clinicians may not be as attentive when examining patients thought to be low risk.

A third shortfall relates to the diagnosis, oral tissue biopsy is usually necessary for lesions that cannot be diagnosed on the basis of the history and clinical findings alone. Approximately ***10% of patients who are examined will have some abnormality of the oral mucosa***. Biopsy is often the definitive procedure that provides tissue for microscopic analysis when additional information is required to guide any indicated therapy.

The development of a reasonable differential diagnosis is of prime importance in determining if biopsy is indicated. Furthermore, the differential diagnosis aids the clinician in selecting the appropriate technique if biopsy is necessary.

A waiting period of 2 weeks often helps in forming the differential diagnosis; lesions that are related to infection, inflammation, or local trauma may resolve during this time. Biopsy is strongly recommended for the evaluation of most lesions that persist for 2 weeks or longer after the potential irritants are removed.

Table 2. Response to the item addressing the reasons for not performing biopsy, or for performing biopsy only occasionally, with the average number of years of professional experience.

Reasons for not performing biopsy, or for doing so only occasionally	Responses (%)	Mean number of years of professional experience (standard deviation)
Lack of experience	52 (34.9%)	8.7 (7.1)
No lesions noted	35 (23.5%)	11.3 (11.4)
Lack of confidence in interpreting the results	11 (7.4%)	8.5 (4.6)
Lack of material	20 (13.4%)	9.3 (7.9)
Others	31 (20.8%)	10.1 (7.5)
Total	149	9.9 (8.6)
Note: these are 5 multiple response alternatives, as a result of which there are more responses than professionals for this item.		

Conventional cytologic examination of the oral cavity is associated with an unacceptably high false-negative rate. Studies have shown that only 21% of dentists in the UK carry out oral biopsies with less than 12% in Northern Ireland.

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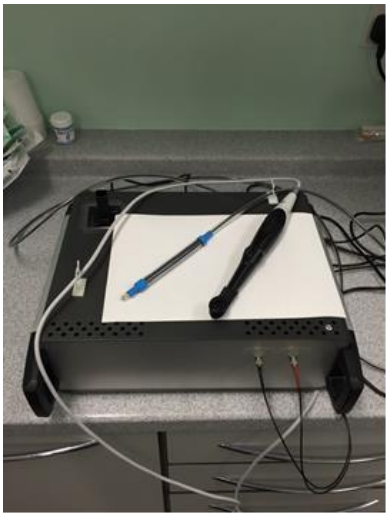
Lastly our research indicates that the fastest growing segment of the oral and oropharyngeal cancer population are otherwise healthy, non-smokers in the 25-50 age range. This is linked to HPV, the human papillomavirus, HPV16 is the version most responsible, and affects both males and females. Every day in the US, about 12,000 people ages 15 to 24 are infected with HPV. In the oral/oropharyngeal environment, HPV16 manifests itself primarily in the posterior regions such as the base of the tongue, the back of the throat, the tonsils, the tonsillar crypts, and tonsillar pillars.

Finally and probably the greatest shortfall in the diagnosis of oral cancers is that for patients, in particular those with no history of oral cancer, present with four or five unique areas of concern, it is unlikely that he or she would readily consent to multiple scalpel biopsies nor would this technique be useful in the non-compliant patient who is unlikely to come back for a follow-up exam or accept an immediate referral to an oral surgeon.

In conclusion, our market research indicates that less than 1 in 4 physicians/dentists can successfully carry out an oral examination, make a differential diagnosis and when appropriate arrange a meaningful referral in a timely manner. These shortfalls are due to lack of emphasis in training, management and diagnostic tools. The fastest growing segment of the oral and oropharyngeal cancer population are otherwise healthy, non-smokers in the 25-50 age range. This is linked to HPV, the human papillomavirus.

The problem in the future will be solved in 3 ways,

- Education of the general public and the physicians' community into the warning signs of oral cancer and how to prevent.
- Diagnostic tools which results in a definitive differential diagnosis and indication for treatment.
- Improved screening methods.



Market Strategy

We have developed a solution for dentists and dental surgeons which will assist them in making a definitive differential diagnosis for oral cancers and improve screening methods. Initially we have chosen to work with dental professionals in Scotland because research has indicated a significant need for

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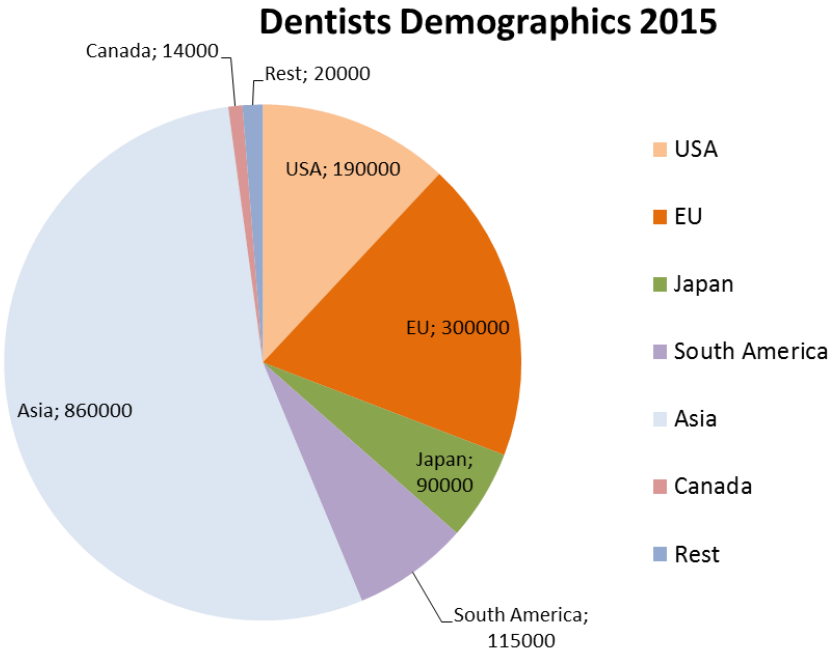
better diagnostic tools and improved screening. Additional clinical sites in the UK have been considers including London and elsewhere in Scotland. Since completion of the project the Academic Hospital in Maastricht has agreed to become the second test site for the demonstrator. The highlights of our strategy are;

- Focus on the European markets (UK is the lead)
- Selecting established distributors for initial revenue.
- Large groups and plans will be targeted first such as the NHS in the UK.
- Our continued partnership with Dundee University and London College will allow us to create clinical data to support application to the NICE (National Institute for Health and Care Excellence).

Market Size

In key markets there are 60 dentists/100,000 population, 190,000 USA, 300,000 EU, whereas in most remaining markets there are 30 dentists/ 100,000 population. Worldwide the number of dentists is approximately 1.6 million. Due to low reimbursement and ROI for clinics only 33% of the developed markets and 10% in underdeveloped markets are accessible. Hence the total accessible worldwide market is 250000 (15%).



Due to cost of ownership (ROI), experience and decision making factors only 25% (62500) of practices will have the means to purchase our product. Given segmentation and operation economics our business can initially only plan on covering 20% of the remaining accessible market, hence our **target market** will be 12,500 (0.8%)

Our strategy over time will focus on the most important markets of which USA and Europe account for more than 2/3's. Asia and South America are remaining key markets. See graph of forecasted markets in 2020.

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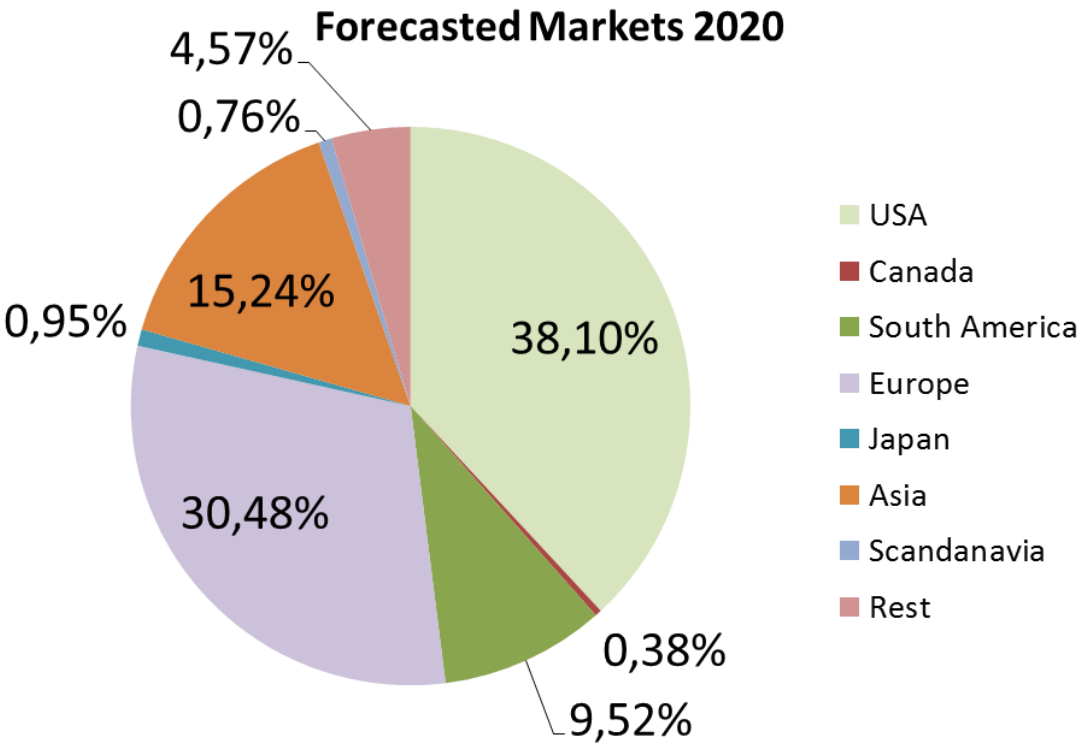

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3.6 WP8 Dissemination and Exploitation

3.6.1 Description of Work package 8

During the project we will make information about the results available via the website. We will go to three conferences/seminars and three relevant trade shows where we will exhibit the Demonstrator.

These contacts will be further exploited in order to benefit the European economy, specifically the participating SMEs and society as a whole in this work package. All production will be done in Europe (PL, UK and NL). In parallel with the industrialization 2M will assess the necessary medical certification required for the product and prepare for CE certification of the device. The resulting Demonstrator will be made available for test by interested parties

We will exhibit the Demonstrator at the Medica in 2015 to both show the results to the medical community and gain further feedback from practitioners on other cancer types.

3.6.2 Dissemination

The website to report on the progress of the project was set up in the first month.

A number of presentations have been given using the EDOCAL Demonstrator both to industrial parties as well as to medical practitioners. The list is included in the on line reporting.

TopGaN presented a paper on EDOCAL at Photonics West and a second paper has been accepted for Photonics West in February 2016 and will be presented by Dr. Macluskey. CSTG has also submitted a paper for Photonics West in 2016

At the last consortium meeting the partners agreed to go for a very focussed dissemination of the EDOCAL system starting in the UK. The system will be introduced to all first, second and third year dental students in Scotland as part of its inclusion in the education plan by Dr. Macluskey and Dr. Theaker.

The system is being introduced to a Clinical focus group for Dentists, who have already signed up as having a particular interest in innovative products for dentistry.

3.6.3 Exploitation

2M has completed the preparation for the CE Certification and is working with KeyTec (Sittard, NL) on the industrialisation. The intention is that the final assembly of the device will be done by Keytec. Keytec was involved in the preparation for industrialization from the beginning of 2015 in order to optimize the production processes as early as possible.

Based on the 2nd generation EDOCALD demonstrator, a cost breakdown was made to the contributions of all partners / suppliers in the project, leading to the first estimate of the commercial price. The 2nd generation demonstrator is based on a combination of laser and

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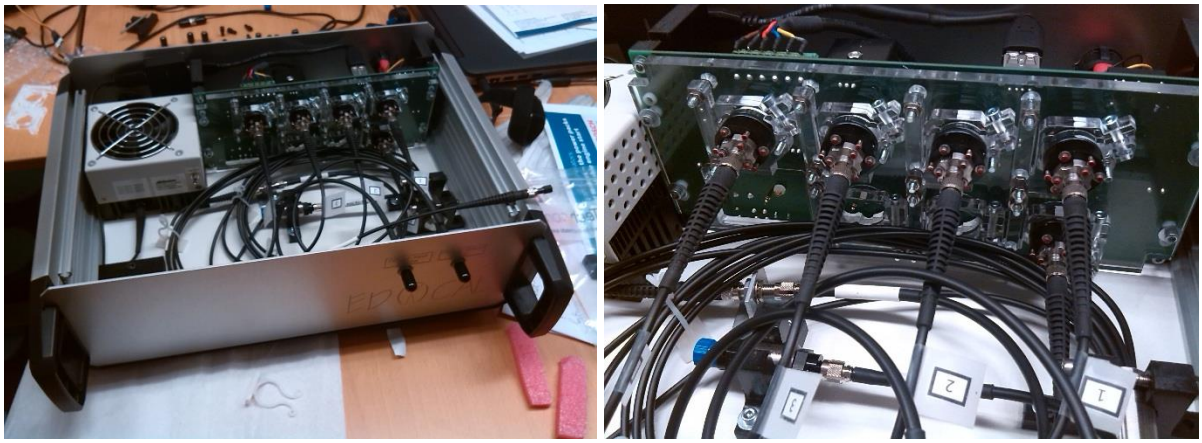
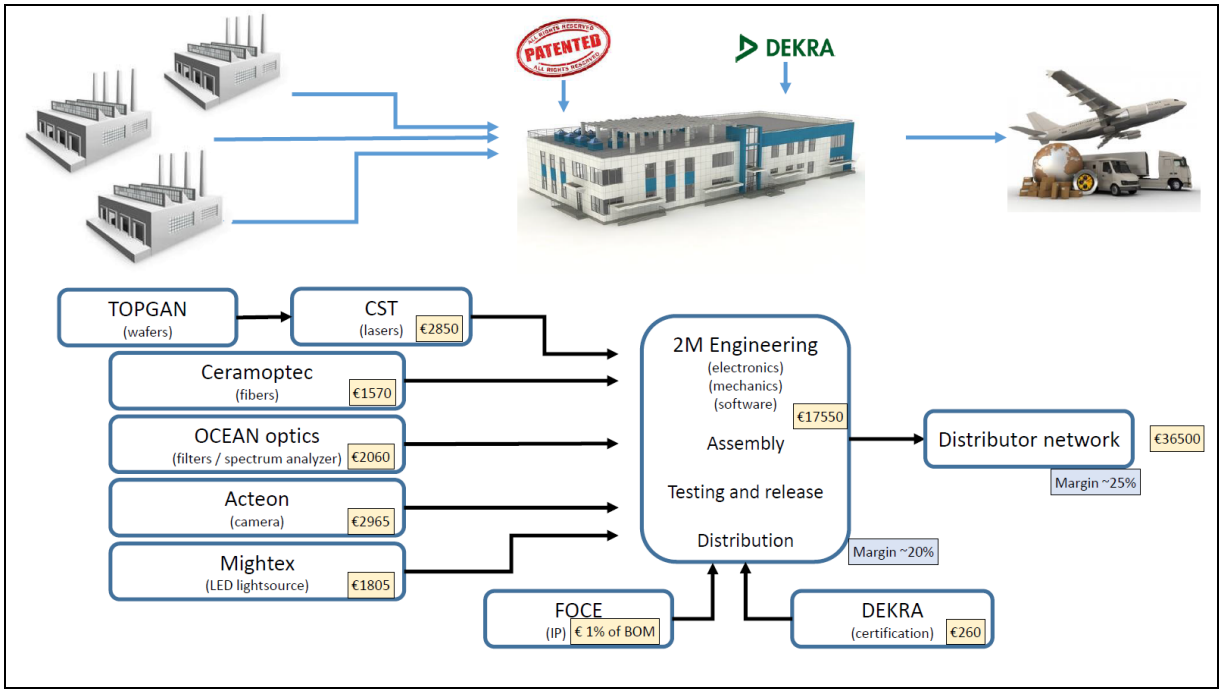
led light sources integrated into a handheld housing, laptop pc with software and dental camera unit.



EDOCALD v2 demonstrator with LED and Laser light sources

In order to exploit this product we plan to set up a separate company called MEDOCAL so that this company can completely focus on the sales of the product. This company will be supported by all of the partners in the consortium and by a number of selected suppliers of key components. The MEDOCAL Business plan is a living document, two versions have been submitted and the document is underging continuous additions and improvements.

The total manufacturing chain is depicted in the figure below:



Current EDOCALD v2 demonstrator supplier breakdown

The main subcontractors are optical component suppliers, providing the different light sources and optical sensors. Mechanical and electrical components are added by 2M Engineering where the total system is built-up into the final product. 2M completed the business plan for a new company “Medocal B.V.”, the company will be formed after more

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patient data is collected and analysed. Further information on the exploitation is contained in D8.6 and is not included here for confidentiality reasons.

3.7 EDOCALD Objectives Addressed

3.7.1 Summary of objectives

Our core objective is to deliver an optical imaging device which creates an image of the oral cavity (broad field white light detection) and maps within the oral cavity highly suspicious lesions with performance targets of >95% sensitivity and specificity using our narrow field laser detector. The output of our Demonstrator will provide not only the image of the suspicious lesions but also their spatial co-ordinates within the cavity (saved as a digital file). Optionally we will offer a means for the dentist to shine the map back into the cavity at any time in order to further evaluate progression after a few weeks or in order to take a biopsy.

3.7.2 Status of objectives

All of the objectives defined in the project plan have been addressed. The three demonstrators have been built and tested in the pre-clinical setting as well as the clinical setting.

The system creates images of the oral cavity and stores them in the database so that the new images can be mapped onto the older images in order to track the progression of the lesion.

The systems contain all of the wavelengths originally planned to be incorporated and the spectra of the measurements are archived in the database along with spectra of healthy tissue from the same patient.

We have added blue light imaging to the system along with the originally anticipated which light imaging. This provides an extra tool for the clinicians who have confirmed that the lesions are much clearer to the naked trained eye under blue light than under white light.

The system is able to distinguish between malignant and healthy tissue with 100% sensitivity and 100% specificity. The conclusion as to whether the system is able to distinguish between pre-malignant tissue that will become malignant and per-malignant tissue that will not become malignant is as yet unknown. This has to do with the number of patients that have been tested positively to date. In general patients who have already been diagnosed positively are not inclined to participate whereas those who still have to be diagnosed are far more inclined to participate. We have to collect date from a far larger group of patients in order to be able to decide on the usability for the system for pre-malignant cancer diagnosis.

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4 Potential Impact, Main Dissemination Activities and

4.1 Potential impact

Early detection of cancer saves lives, improves quality of life and reduces healthcare cost.

“One third of the cancer burden could be cured if detected early and treated adequately. Early detection of cancer is based on the observation that treatment is more effective when cancer is detected earlier. The aim is to detect the cancer when it is localized……..Fundamental for adequate treatment is an accurate diagnosis by means of investigations……..” a quote from the World Health Organisation.

In Europe there are 3 million new cancer cases per year (38 countries), with 2 million new cases in the EU25 alone. This represents 340 new cases / 100000 people per year. One in three men and one in four women will be inflicted with cancer during their life. There is no doubt about the fact that cancer is an increasingly important factor in the global burden of disease with tremendous economic impact. The 11th most common form of cancer is Oral cancer. This effects men and women alike. Oral cancers tend to be detected and diagnosed late in their development, leading to a maximum life expectancy of only 5 years after diagnosis for almost half of the patients. Currently there is no current solution commercially available for routine screening,

The commercialisation of the results of EDOCALD will have far reaching effects not only economically but also socially, and not only in the developed world but throughout the entire human race. One of the reasons, is that the technology we are using for the core components is low cost and mass producible meaning that the price of the final products should make them accessible to all.

Not only will cancer patients and their families benefit from the results of EDOCALD, additional benefits exist in the form of huge reductions in medical costs. New research results published on April 26th, 2012 by Delta Dental of Michigan's Research and Data Institute (RDI) finds that in the United States, the cost of oral cavity (OC), oral pharyngeal (OP), and salivary gland (SG) cancer may be the most costly cancer to treat. It concluded that on average, total annual health care spending during the year following diagnosis was $79,151 compared to $7,419 in a group comprised of similar patients without these cancers. The research also determined that the average cost of care almost doubled when patients received all three types of treatment including surgery, radiation and chemotherapy. Most oral cancers require costly and disfiguring medical intervention, and even then the five-year survival rate is less than 60%. Yet when the cancer is detected early, the survival rate increases to 83%.

A further benefit is that the product provides the medical community with a practical tool that can easily and conveniently be used in day to day medical practice, allowing them to do their work quicker as fewer biopsies are needed and the development of lesions can be recorded objectively. This improves the quality and accuracy of their work as they will now have the tools needed to discriminate cancerous from healthy tissue at the point of care. A news article about the United States suggested that 85% of all biopsies taken are unnecessary as the cells turn out to be healthy. A tool that can reduce the need for many types of biopsies will lead to enormous and highly necessary cost savings for health care which has seen

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dramatic changes in recent years due to the ever increasing number of cancer and diabetes patients. During the EDOCALD project we have developed demonstrators for systems that ca be used to track the development of the lesions providing objective information to the clinicians. Currently we are still testing the sensitivity and specificity of pre-malignant tissue. Our intention is not to commercialise one product but a series of products starting with the imaging system and moving to the diagnostic system as more patient data is accumulated.

4.2 Main dissemination activities

At the beginning of the project we set up the website that is used to host the project information and also the information we want to make available to the broader public about the project. The website therefore has a secured part for project participants that requires a login password and a public part which can be viewed by all. The latter will be used to raise the image of EDOCALD and improve dissemination to specialists, potential users of the technologies being developed, politicians and public funding authorities, as well as the general public.

Keywords are used on the home page that will enhance the hit rate from Google searches. Hyperlinks will be made to and from the participant companies. Simple document structures will be used and mostly in A4 length. On the homepage short progress statements and news items are updated regularly.

4.3 General broad dissemination of the results

UNIVDUN has completed the validation of the system with positive results. It is the intention of all of the beneficiaries to continue working on data collection from a larger number of patients and to extend the data collection to more sites (University college London who has shown interest from the beginning will be the first.). Two academic hospitals in the Netherlands have shown interest be being test sites for collecting patient data and discussions with them are underway.

Professor Mossey is in discussion with Cairo University Dental Hospital & School, Professor Amr Abou-El-Ezz, Professor of Orthodontics and Vice Dean for Graduate studies and Research in a Dental School Department that sees up to 50 new cases of oral cancer at various stages of progression or pre-canerous lesions per week. They are in the process of setting up a programme of research with the University of Dundee Dental School, and they are interested in the EDOCALD concept.

A first presentation of the results was given by TopGaN at the Bios at Photonics West last February. Two papers have been submitted for the coming Photonics West Congress. CSTG has submitted an abstract on the laser technology used and UNIVDUN Dental School will present a paper on the results of using the demonstrator.

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4.4 Dissemination in a focused way.

Dr. Macluskey and Dr. Theaker from UNIVDUN Dental school are part of a Dental clinical focus group specifically interested in white lesions. The group consists of 7 experts, who arrange to show new innovative products in dentistry to groups of dentists interested in innovations. EDOCALD and the results of the clinical study will be presented to these groups.

Dr. Macluskey and Dr. Theaker are also involved in an annual initiative form 1,2 and 3rd year Dentistry students. The next meeting will be held on October 28th, 2015 and the EDOCALD system will be demonstrated there.

Once sufficient data has been gathered (200+ patients) papers will be submitted to the following medical congresses:
1. British Dental Conference and Exhibition (2016)
2. WFLD - World Federation of Laser Dentistry (2016)
3. Greater New York Dental Meeting (27th Nov. 2016)

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trade shows we will be targeting are:
1. MEDICA (Germany, November, 2016)
2. British Dental Conference and Exhibition (2016)
3. Greater New York Dental Meeting (2016)

We plan to arrange papers and presentations for other Dental organisations in order to promote and educate patient groups about EDOCALD in particular the ADA and the BDA.

We are currently making the results known to the medical community by exhibiting at medical congresses and seminars. The presentations there will be done by UNIVDUN and potentially medical centres who agree to become the early adopters of the EDOCALD system and support filling the Lesion and Image databases. The process of dissemination will continue as part of our exploitation activities.

These activities will include the formation of a Clinical focus group and a Scientific Advisory Board made up of medical professionals whose job includes the uptake and creation of a dissemination of innovative dentistry products. Since the entry market will be Dental we will have 3 Dental professionals initially on this board.

These plans will include;

- Equipment training as part of course work in Dental/Dental Surgeon educational institutes, the first steps towards this will be taken at the annual focus group initiative on October 28th, 2015
- Market material for distributors and direct mail to end-users;
- Training days/sessions at large clinics and congresses;
- Collaborations with important Research groups to continue the validation of this project and evaluation of our project for use in additional applications.

One publication has been made and two more are planned for February 2016 at the Photonic West Conference.

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4.5 Exploitation of Results

Based on the 2nd generation EDOCALD demonstrator, a cost breakdown was made to the contributions of all partners / suppliers in the project, leading to the first estimate of the commercial price. The 2nd generation demonstrator is based on a combination of laser and led light sources integrated into a handheld housing, laptop pc with software and dental camera unit.

EDOCALD v2 demonstrator with LED and Laser light sources

In order to exploit this product we plan to set up a separate company called MEDOCAL so that this company can completely focus on the sales of the product. This company will be supported by all of the partners in the consortium and by a number of selected suppliers of key components. The MEDOCAL Business plan is a living document that is underging continuous additions and improvements.

The total manufacturing chain is depicted in the figure below:

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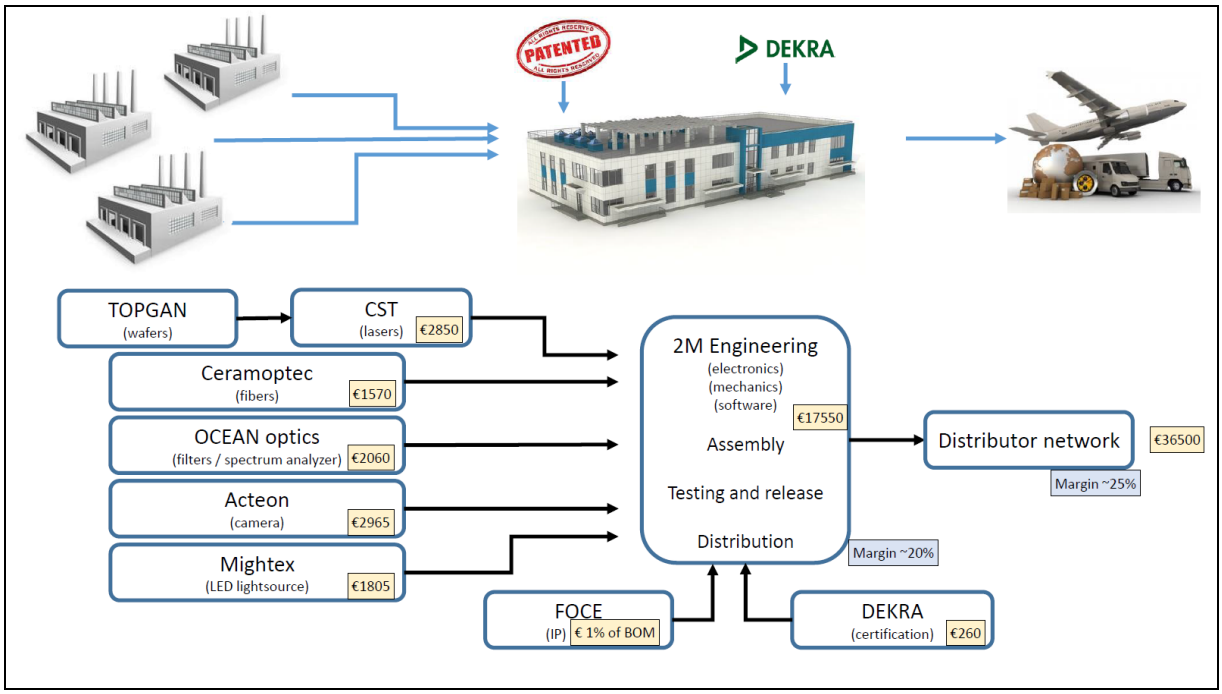


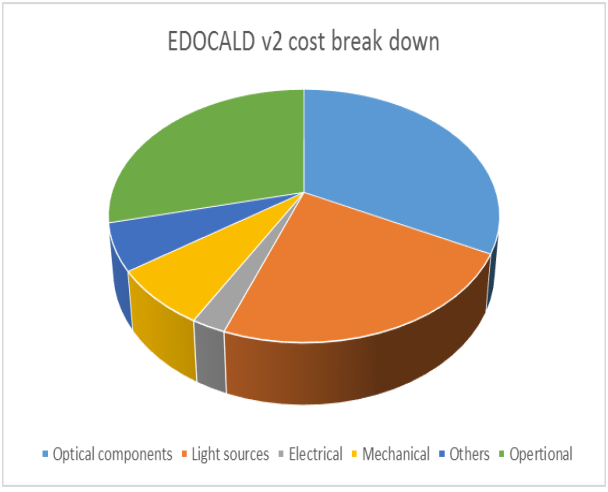
Current EDOCALD v2 demonstrator supplier breakdown

The main subcontractors are optical component suppliers, providing the different light sources and optical sensors. Mechanical and electrical components are added by 2M Engineering where the total system is built-up into the final product.

IP royalties and certification costs are covered as separate components and we include the costs for assembly, testing and distribution is order to come to a first estimate of the commercial price.

Taking into account that product distribution will not be done by 2M Engineering, but by partners who are already established in this field, the margin added for these distributors gives us a first rought estimate of the product price at market introduction.

The price is significantly higher than the price we are targetting and a significant cost down step will be needed.



The total cost breakdown shows that the main cost component is in the total optical system.

The amount and type of light sources will dictate the main cost of the final product. The cost is composed of two components:

- Number of light sources
- Type of light sources needed

The amount of light sources must be defined by testing, for the different types of light sources a cost comparison between LED and Laser has been made.

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For the EDOCALD v2 system there a 3 different light source possibilities:

1) 5 Lasers + 3 LED light sources
2) 8 up LED light source
3) 2 x 4 up LED light sources

Current procng information shows that the cost saving on the mechanical and electrical components will be around € 500, reducing the price difference between the LED and combined Laser / LED solution to €1000.

When ordering higher volumes the difference will become smaller:

- 1 piece order, full LED solution = + € 1000 component cost
- 10 piece order, full LED solution = + € 400 component cost
- 100 piece order, full LED solution = comparable, assuming also Laser cost will reduce at higher volumes

The main advantage of the LED solution is it current availability and the maturity of the technology. The 2x4 LED design can be made and implemented today, while the laser solution will depend strongly on the availability of the substrate materials needed to make the lasers. The cost for LEDs could be lower for higher volumes, this is currently under discussion with the supplier as they are not accustomed to supplying their led modules in large quantities.

With the current information that is available, the final choice for LED or Laser / LED light sources has no big direct influence on the estimated final product cost.

Medocal plans to develop an important strategic alliance with a large, more established business in the endoscope market, we are targeting the top 3 in Europe. We plan to conclude a technology license which will be for endoscopic applications only and exclude the Dental and Neck markets. Medocal will receive milestone payments in the first 3 years and royalty payments for products sold. Together we will develop a solution for one type of endoscope.

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We have a strategic relationship with a OEM manufacturer. Keytec B.V. is a medical device OEM who is medical certified and can arrange all the necessary certifications.

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