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Infrared spectroscopy based on broadly tunable quantum cascade lasers and polycrystalline diamond waveguides

Recently emerging broadly tunable quantum cascade lasers (tQCL) emitting in the mid-infrared (MIR) are a versatile alternative to well established thermal emitters in combination with interferometers as applied in Fourier transform infrared (FTIR) spectroscopy. Combination of tQCLs with durable optical transducers, i.e. polycrystalline diamond as waveguide for attenuated total reflection (ATR) based spectroscopy opens possibilities to analyse the composition of biological and medical samples as complex as body fluids. The high-energy output of tQCLs in the MIR enables elongated optical path lengths, aka interaction path lengths with the potential to reliably detect minute analyte amounts.
Infrared spectroscopy based on broadly tunable quantum cascade lasers and polycrystalline diamond waveguides

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Recently emerging broadly tunable quantum cascade lasers (tQCL) emitting in the mid-infrared (MIR) are a versatile alternative to well established thermal emitters in combination with interferometers as applied in Fourier transform infrared (FTIR) spectroscopy. The wide and highly spectrally resolved wavelength tuning characteristics along with superior spectral energy density renders laser-based vibrational spectroscopy methods an efficient alternative vs. conventional molecular spectroscopies. Using diamond in attenuated total reflection (ATR) sensing formats benefits from the physical robustness and chemical resistance of the internal reflective element (IRE) material. While inherent material absorption frequently limits the optical path length within diamond ATR elements, the herein presented design combining bright tQCLs with a multi-reflection polycrystalline diamond (PCD) ATR element enables an optical beam path length of approximately 5 cm. Thereby, sensitive spectroscopic measurements in the MIR are enabled. As an example, non-invasive glucose monitoring in human saliva is examined, highlighting the potential benefits of the proposed analytical concept with regards to exquisite sensitivity and selectivity in combination with a robust sensing interface, i.e., diamond. This approach paves the way towards directly analyzing molecular constituents in complex and potentially corrosive biomedical and biochemical matrices.

Introduction

Analysis in the mid-infrared (MIR) (2.5 µm to 25 µm, 4000 cm−1 to 400 cm−1) is a spectroscopic strategy routinely deployed in a wide field of scenarios ranging from process analytics to biomedical applications.1 However, limitations include the required relatively high analyte concentrations, rather high sample volumes in the mL range or above, and comparatively bulky instrument dimensions. Routinely used thermal emitters such as SiC globars provide a limited spectral energy density in the MIR, which directly relates to sensitivity limitations especially in strongly absorbing analyte matrices. However, recent progress in broadly tunable laser light source technology provides a promising alternative vs. established non-coherent light sources, as a significantly higher energy density per wavelength is intrinsically provided.

The introduction of quantum cascade lasers (QCLs) operating in the MIR has rendered this technology especially interesting for (bio)chemical analysis and sensing tasks. Notably, two different approaches are being realized with QCL technology. On the one hand, high resolution can be achieved with distributed feed-back (DFB) technology, yet at the cost of narrower tuning ranges. On the other hand, external cavities (ECs) provide broader tuning ranges albeit at lower spectral resolution. Lately, QCL systems providing high resolution in combination with relatively broad tuning ranges (155 cm−1) have been introduced using EC architectures.2 Nowadays, QCL spectrometers are available providing tuning ranges up to 1100 cm−1 via up to four individual lasers3–5 covering about 270 cm−1 each operated in parallel, and providing up to 500 mW of pulsed or continuous wave (CW) laser radiation. With increasing availability, QCL technology has already been used for detecting a variety of medical analytes.6 For instance, the detection of trace amounts of cocaine has been demonstrated.7,8

Attenuated total reflection spectroscopy (ATR) makes use of an exponentially decaying evanescent field emerging at the surface of a waveguiding total internal reflection element (IRE) and is considered a complementary sampling strategy vs. transmission techniques. As an IRE or waveguide, high refractive index (RI, n) materials are required, which are ideally also chemically and physically resilient. Diamond combines these
favorable properties and is therefore among the favored ATR element materials. However, single crystalline diamond (SCD) remains quite expensive to fabricate and process. In contrast, polycrystalline diamond (PCD) offers comparable performance in the MIR at approximately one tenth of the fabrication costs. Conventionally, the limited power output of standard MIR sources and the intrinsic optical absorption of diamond limits the propagation path length through diamond ATR elements, at which acceptable signal-to-noise ratios (SNR) can be achieved. This is the reason, why diamond ATR elements are predominantly configured as single internal reflection elements.

The reduction of bulk diamond crystal dimensions to thin-film waveguides and the introduction of more powerful MIR laser sources nowadays enables overcoming this limitation. Thin-film diamond waveguide technology in combination with laser sources has already been shown for the detection of organic compounds and proteins in the MIR.9–11 In these contributions, free-standing core-only (i.e., air-clad) diamond thin-film waveguides supported by a silicon frame have demonstrated their utility for analytical applications. However, processing limitations including high strain within the deposited diamond layers, thermal expansion, and demanding processing parameters at elevated temperatures in a hydrogen atmosphere compete with matching the required optical parameters such as the refractive index. Thin-film diamond growth on silicon substrates with subsequent partial removal of the supporting substrate is a tradeoff between stable growth conditions and refractive index matching at the expense of potentially more elaborate processing opportunities for waveguide fabrication. Although free-standing diamond structures are self-supporting, they remain brittle in nature and are therefore mechanically sensitive, which is not an issue for bulk diamond IREs.

The physical and chemical resistivity, biocompatibility, and autoclavability of diamond renders analytical concepts based on diamond ideally suited for bioanalytics. Body fluids are a rich and well-known source of biomarkers relevant in medical diagnostics and for clinical monitoring. In particular, non-invasive alternatives to blood or spinal fluid are more convenient for patients and less prone to potential complications such as inflammation due to the puncture. Even tear fluid, sweat or urine samples are inconvenient to obtain. With globally increasing diabetic rates, non-invasive glucose testing is of increasing interest, and has become a driving force in the development of non-invasive analytical devices.12,13 Exemplarily, non-invasive blood glucose monitoring has been presented by the research group of Mäntele utilizing QCLs and photoacoustic cells. Detection of <50 mg dL⁻¹ up to >300 mg dL⁻¹, which is in the clinically relevant regime has been achieved.14,15 Furthermore, non-invasive electrochemical determination of glucose in human saliva has been presented.16 In general, saliva appears to be a readily obtainable human body fluid suitable for medical diagnostics,17 comparable to expired human breath.18 Exhaled breath analysis has matured into a frequently applied strategy to obtain CO₂ and volatile organic compound (VOC) concentrations, while saliva comprises a certain percentage of condensed breath constituents. Furthermore, buccal spectral markers have been found to correlate to potential lung cancer risks.19 Even the detection of α-synuclein serving as a potential biomarker for Parkinson’s disease in cheek cell samples has been shown.20 Likewise, simple molecules such as salivary uric acid are non-invasively addressable biomarkers for metabolic syndrome.21,22 Even physiological stress has been shown to correlate with changes in the salivary composition, and the associated MIR spectral features.23 Infrared spectroscopy has also been applied to detect protein shifts of psoriatic and diabetic patients in saliva.24 Notwithstanding, a potential spectral correlation between blood glucose and salivary glucose has been proposed in literature.25,26 Biologically relevant concentrations of glucose in saliva are reported to range from 0.008 mg mL⁻¹ to 0.0105 mg mL⁻¹ (ref. 16 and 27) in healthy patients, and from 0.04 mg mL⁻¹ to 0.14 mg mL⁻¹ for diabetic patients.28 This is approximately one tenth of the concentration range expected in blood extending from 0.88 mg mL⁻¹ to 9.4 mg mL⁻¹.28 Hence, improved sensitivities especially for IR sensing schemes are required for glucose analysis in saliva.

In the present study, an IR spectroscopic analysis system based on a QCL in combination with a PCD IRE has been developed. The utility of the system for addressing the MIR fingerprint regime enabling non-invasive saliva glucose level analysis is evaluated.

Experimental method: MIR evanescent field sensing system based on a PCD optical transducer

Saliva sampling

Approx. 1 mL of saliva was sampled from a healthy volunteer, between 20 and 30 years old (BMI 26.7) per trial by unstimulated drooling. Prior to sampling, no food or beverages were allowed for one hour to avoid interference of residual food remains in the oral cavity such as proteins, fat or hydrocarbons with the subsequent analysis. Directly before sampling, present saliva was swallowed and the oral cavity was rinsed with approx. 50 mL of mineral water ensuring recently produced saliva. For time-dependent analyses, samples were collected every 10 min.

The saliva samples were obtained according to the Declaration of Helsinki.29 The healthy volunteer was instructed orally and by writing and has given written consent.

Experimental QCL setup

The experimental setup (Fig. 1) is based on a broadly tunable mid infrared quantum cascade laser system (MRcat, Daylight Solutions). The laser head comprises of four individual broadly tunable quantum cascade lasers, optically coupled into one single output port. Thus, a spectral range coverage from 4.95 μm to 11.2 μm (20 cm⁻¹ to 890 cm⁻¹) is provided at a peak output power (i.e., at the center wavelength of each
beveled in- and out-coupling facets at an angle of 45° and a
ground spectrum (\(A\)) and absorbance (\(I_0\)) were recorded across the entire tuning range of the laser system,
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Results and discussion
Evaluation of the optical properties
The utilized PCD with a surface area of 15 × 12 mm² with beveled in- and out-coupling facets at an angle of 45° and a

PCD characterization
The spectral characteristics of PCD in the MIR were evaluated using an IFS 66 v/s FTIR spectrometer, and were compared to a SCD specimen of the same thickness (approx. 450 µm). The spectrometer was equipped with a room-temperature DTGS (deuterated \(\alpha\)-alanine glycine sulfate) detector (Bruker Optik GmbH). Data was recorded via the OPUS software package (Build 6.5, Bruker Optik GmbH) at a spectral resolution of 4 cm⁻¹ in the range of 4000–600 cm⁻¹ (2.5–16.7 µm) averaging 32 scans for each spectrum. The diamond samples were mounted within a transmission holder in order to propagate the IR beam through the 450 µm thick material, i.e. perpendicular to the beam direction used for QCL ATR measurements. Finally, data was evaluated via Origin (OriginPro 2017G, OriginLab). As shown in Fig. 3, no significant difference between SCD and PCD diamond in grain growth direction was apparent. While weak Rayleigh scattering may still occur, it should be negligible towards longer wavelengths following \(\sim 1/\lambda^4\) within the crystal boundaries of the IRE.\(^{30,31}\)

Furthermore, interference with sp² hybridized graphitic carbon at crystallite boundaries appears negligible. It should be noted that non-sp³ hybridized carbon atoms within the first few deposited PCD layers have been removed by polishing. Inherent diamond lattice vibrations (i.e., two-phonon absorptions) are however present, and render IR studies in the spectral region of 2600–1500 cm⁻¹ of limited utility due to the reduced energy throughput.
Using a QCL light source, higher spectral energy density is achieved, which renders the spectral regions close to the two-phonon absorption features still useful for analytical applications. As shown in Fig. 4, parabolic emission curves of the four applied laser crystals with intensity maxima around 1900 cm\(^{-1}\), 1600 cm\(^{-1}\), 1350 cm\(^{-1}\), and 1100 cm\(^{-1}\) (5.25 µm, 6.25 µm, 7.5 µm, and 9 µm, respectively), are superimposed by the diamond lattice absorption emerging towards the shorter wavelength regime. Notably, transmission is still achieved up to 1800 cm\(^{-1}\) although IR radiation has already been propagated approx. 5 cm through the trapezoid PCD IRE structure.

**Optimization of the laser light source**

Due to pulsed operation of the QCLs, intensity deviations in between subsequent pulses had to be efficiently suppressed, which is commonly achieved by averaging a sufficient number of subsequent pulses. Using an Allan deviation experiment, the most suitable number of averaged laser pulses has been determined.\(^{32-34}\) Resulting, 10 000 subsequent laser pulses were recorded, and their Allan variance has been plotted (Fig. 5). Consequently, 500 subsequent pulses were averaged during all further experiments as a trade-off between noise reduction (i.e., Allan deviation <10\(^{-3}\)), and adequately short measurement times. Furthermore, 5-point adjacent-point-averaging was performed on the retrieved spectra to reduce inter-wavelength intensity deviations arising from the step-wise tuning of the lasers.

**Analysis of human saliva**

Human saliva analysis was selected as an application example due to its accessibility, while constituting a real-world complex biological matrix.\(^{35}\) In human saliva, various organic molecules are present resulting in distinct fingerprint absorption bands in the MIR, i.e., hydrocarbons, fats (\(\sim\)1100 cm\(^{-1}\)), and proteins, i.e., around 1650 cm\(^{-1}\) assigned to the C=O stretching mode (amide I band), and around 1550 cm\(^{-1}\) assigned to a N–H bending mode (amide II band). Furthermore, symmetric and asymmetric stretching mode bands of carboxylate (COO\(^{-}\)) are present around 1400 cm\(^{-1}\) (Fig. 6). The latter can be assigned to lactic acid molecules or protein side chains with changes evident when comparing diabetic and non-diabetic patients. In an exemplary study, glucose levels in a healthy volunteer were evaluated. A distinct band at 1030 cm\(^{-1}\) was
selected for the glucose determination within the MIR spectrum of saliva, which represents the CO vibrations of the glucose molecule.36

Per analysis, the obtained saliva samples were divided into equal aliquots, and spiked with aqueous solutions of alpha(+) D-glucose (\(M = 180.16 \text{ g mol}^{-1}\), VWR International GmbH) for establishing calibration samples via standard addition. Subsequently, 20 µL of each solution were transferred with an Eppendorf Pipette onto the PCD ATR IRE and the aqueous matrix was left to evaporate. A volume of 20 µL was determined to cover the surface of the diamond crystal, while avoiding spills affecting the in- and outcoupling facets. However, introducing an appropriately sealed liquid cell is supposed to enhance the reproducibility during future experiments by reducing variances resulting from viscosity variations of the saliva sample.

After each analysis, the crystal was cleaned with water, acetone, and isopropanol/lens cleaning tissue, which effectively removed the previous sample. The spectral region of interest within the obtained spectra for glucose determination was limited to 1200–900 cm\(^{-1}\). The spectral region was smoothed with a 0.02 Hz low pass FFT filter, and subsequently normalized. In a next step, peak deconvolution was performed using Lorentz shaped bands fitted to the spectra. The peak centered at 1030 cm\(^{-1}\) characteristic for glucose was then integrated for quantification. An exemplary peak fit is shown in Fig. 7 for an unspiked sample, and for a sample spiked with 0.17 mg mL\(^{-1}\) glucose.

With the developed experimental procedure, a limit of detection (LOD) of 0.02 mg mL\(^{-1}\) was derived using the 3\(\sigma\) noise criterion. This LOD is close to biologically relevant concentrations derived from literature for saliva ranging from 0.008 mg mL\(^{-1}\) to 0.0105 mg mL\(^{-1}\) (ref. 16) for baseline values of healthy patients, and is well below the expected values for analyzing saliva glucose levels in diabetic patients ranging from 0.04 mg mL\(^{-1}\) to 0.14 mg mL\(^{-1}\).

Time-dependent variation of saliva glucose levels was evaluated on a healthy volunteer between 20 and 30 years old (BMI 26.7). After one hour of baseline monitoring, 500 mL of regular Coca Cola (Coca Cola Company) were consumed to deliberately rise saliva sugar levels (i.e., 10.6 g of carbohydrates – mainly sucrose – per 100 mL).

Fig. 8 shows the time-dependent development of saliva glucose levels. Within the first 60 min, the saliva glucose level shows a variance between 0.05 mmol L\(^{-1}\) (0.01 mg mL\(^{-1}\)) and 0.35 mmol (0.06 mg mL\(^{-1}\)) around an average of about 0.2 mmol L\(^{-1}\) (0.04 mg mL\(^{-1}\)). This elevated variance is explained by variations of the saliva production itself and potential manual sample handling issues and have to be further substantiated during future studies. For example, inter-sample variances may be addressed by the addition of an internal standard. Deviations in saliva flow, resulting amount of saliva, and viscosity, as well as tissue or gland aspects may be addressed as well using this procedure. A slight decrease of
Increased number of patients has to be executed for evaluating research on improved evaluation algorithms and with performance for analysis in a complex real-world matrix, further been selected as an example demonstrating the device performance already at an earlier stage.

Conclusions and outlook

The combination of a broadly tunable QCL assembly serving as a bright mid-infrared light source in combination with polycrystalline diamond ATR IRE has been shown. The latest generation of tQCLs provides high spectral energy density across a broad and customizable spectral window, as required for target-specific device design or broadband analyte detection.

Author contributions

All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

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Notes and references


