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Towards infrared photonic integrated circuits (PICs) in biochemical analysis: implementation of quantum cascade lasers (QCLs) in analysis of fluids

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Infrared spectroscopy in analysis of wastewater and in bioreactor applications: from FTIR to quantum cascade lasers (QCLs)

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Abstract

There is a growing demand for hand-held and/or field-grade sensors for biochemical analysis of fluids. These systems have applications in monitoring of nitrogen-based compounds (such as nitrate and ammonia) in the wastewater treatment industry; bacterial detection in drinking water; analysis of biofluids, such as urine or blood; and in many other areas. Mid-infrared (mid-IR) spectroscopy is a powerful tool for identification and quantification of a wide range of common organic and inorganic compounds. Although IR radiation is strongly absorbed in water, this technology can be adapted for analysis of fluids by utilizing the principles of attenuated total reflection (ATR). In this contribution we highlight the application of IR spectroscopy in wastewater analysis as well as for metabolomic analysis in bioreactors. We discuss the requirements for IR signal stability that are necessary for biochemical analysis of fluids and provide examples of challenges encountered during transition from FTIR to a QCL-based platform. Overall, our stepwise efforts target eventual integration of a QCL light source, waveguide sensor, and IR detector onto a single photonic integrated circuit (PIC) for applications in the defense sector as well as for a broad consumer market.

I. Introduction

Infrared spectroscopy is a powerful tool for molecular analysis of various substances, including gases, solids and fluids. Max-IR Labs drives application of infrared (IR) spectroscopy in biochemical process control and optimization. Although spectrometers based on Fourier Transform Infrared (FTIR) or tunable quantum cascade lasers (QCL) allow infrared sensing, in-line analysis of fluids is still challenging as it requires engineering of simplified sampling and measurement methodologies.

In this article we will describe several applications of IR for in-line analysis of fluids. The first section will be dedicated to description of a sensor for field analysis of sewage, for application in real-time process control at municipal wastewater treatment plants (WWTPs). The second section will describe the application of IR sensing in environmental bioreactors, through analysis of the bacterial metabolic signature. The third section will present the requirements for IR signal stability in biochemical analysis of fluids. These requirements are critical when considering transition from FTIR spectrometers onto QCL platforms, in particular towards miniaturized photonic integrated circuit (PIC) platforms.

II. Infrared analysis for process control at WWTPs

All modern cities have large-scale wastewater (sewage) treatment plants. At the onset of 19th century, crowded cities suffered from poor sanitation. Sewage, discharged into the surface

waters of local rivers, led to outbreaks of waterborne diseases. For instance, in London, cholera outbreaks in 1832, 1849 and 1854 resulted in high mortality rates due to contaminated water sources¹. This and the Great Stink over the river Thames¹ of 1858 prompted the beginning of a new era, marked by extensive construction of sewage systems and science-driven methods for sewage treatment. The major breakthrough resulted from intensive European and American filtration research² that ultimately led to establishment of the “activated sludge” method of sewage treatment. This method relies on the supply of *oxygen* through the process of *aeration* and simultaneously on preservation of the biomass of microorganisms^{3,4,5,6} in wastewater treatment basins.

While *aeration* is a backbone of modern wastewater treatment to this day, it is an extremely energy-intensive process, which *consumes over 50% of electric energy at WWTPs*⁷. Monitoring of nitrogen-based contaminants in wastewater in the form of nitrate and ammonia can help save over 20% of electric power^{8,9} by avoiding excess aeration. For a WWTP treating water from an average American suburb of 150,000 people, this can translate into savings of roughly 3,000kWh of electric power daily. With over 16,000 centralized wastewater treatment plants in the US alone, potential US electric power savings from implementation of this technology for improved process control amount to roughly 16TWh per year¹⁰.

Max-IR Labs develops sensors to enable detection and speciation analysis of nitrogen contaminants including from nitrate, nitrite and ammonia^{11,12}. The sensor consists of a spectrometer – either QCL-based or FTIR, and a cartridge that consists of an Ion-Selective Material (ISM) and an IR waveguide (the technology is referred to as **ISMIR™**). The sensor outline is presented in Fig. 1A. Details of the ISMIR™ cartridge are shown expanded in Fig. 1B. Measurements are performed using an evanescent field that penetrates into the surrounding ion-selective material (ISM) for sensing of pre-concentrated contaminants.

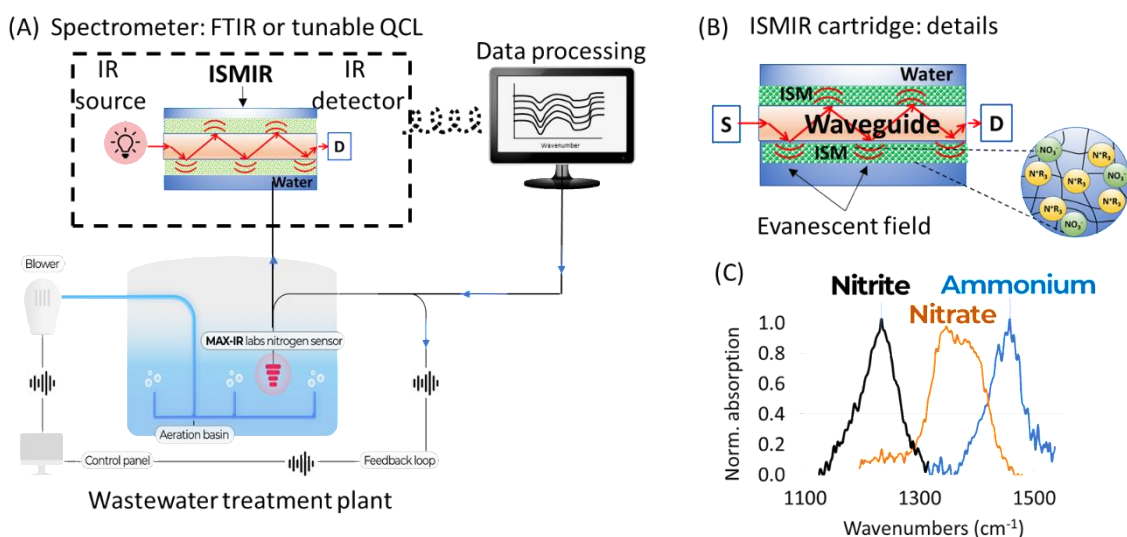


Figure 1 A. Infrared setup for detection of contaminants in water using evanescent field. ISMIR cartridge is coupled with an FTIR or a tunable QCL spectrometer. B. Details of ISMIR cartridge that consists of a waveguide and ion-selective material (ISM). S: source; D: detector. C. IR absorption bands of nitrate, nitrite and ammonium are spectrally distinguishable in the IR spectral range.

The ISMIR™ method is optimized for detection of contaminants in the presence of a complex matrix, such as wastewater, which is rich in multiple organic and inorganic substances. IR spectroscopy enables discrimination of specific contaminants that are recognizable due to their particular absorption bands. The ISM provides an additional layer of selectivity due to preferential absorption of, for example, nitrate ions. Our experience shows that no spectral deconvolution is required when an ISM is employed in the measurements, which improves the sensitivity of this method. The ISM serves a dual role: *pre-concentration* and *selectivity*.

One of the strong advantages of this technology over other nitrogen sensors¹³ such as UV¹⁴ and ion-selective electrodes^{15,16} is the ability to monitor wastewater without frequent calibration, enabling fully autonomous sensing, and minimizing maintenance. Indeed, one of the reasons for the low adoption of sensors at wastewater treatment plants is high operational expenditure (OPEX), mainly due to requirements for frequent sensor calibration and cleaning.

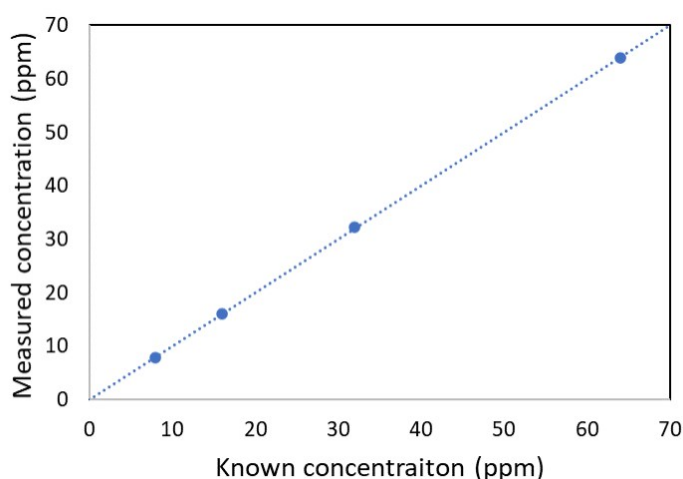


Figure 2 Detection of nitrate in water: concentration measured based on IR signal with ISMIR™ system vs. actual concentration of NO₃-N

The ISMIR™ cartridge can be simply replaced every 3 to 6 months by any personnel without any prior training. Other advantages include speciation to nitrate, nitrite and ammonia, and the possibility to expand sensing to a range of organic and inorganic contaminants.

Fig. 2 shows a calibration chart obtained using ISMIR™ technology for nitrate (the concentration is shown as N-nitrogen), where the low error bars indicate limits of detections down to 0.7 ppm with 99.9% confidence and with a 95% confidence interval of the measured values of +/- 0.4 ppm.

Our first extended in-situ demonstration of the technology at a wastewater treatment plant is scheduled for January 2022, after which the prototype will be productized and commercialized.

III. Bioreactors

Aeration basins at wastewater treatment plants are an example of a specific case of large-scale bioreactors, where multiple microorganisms are involved in decomposition of nitrogen components through a series of aerobic and anaerobic stages. However, application of IR technology in *lab-scale* bioreactors is easier, where appropriate reactor design with integrated infrared elements allows analysis of various microorganisms, from yeast to bacterial growth in urine.

The infrared data obtained from minimal growth media with *P. denitrificans* in anaerobic conditions is presented in Fig. 3. *P. denitrificans* is a model organism for characterization of denitrification processes. The data shows the consumption of succinate and nitrate (shown as “negative” peaks) and appearance of a peak due to N₂O (positive peak).

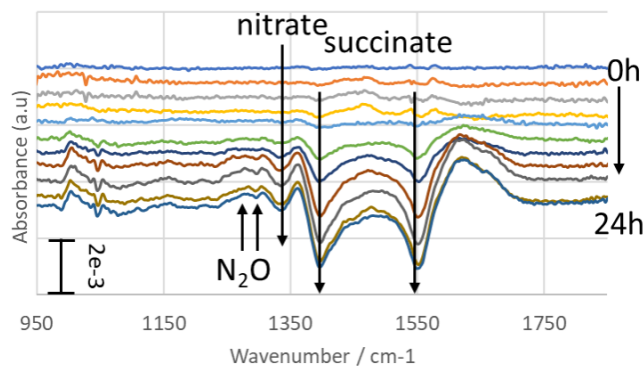


Figure 3 IR spectra of consumed and produced metabolites obtained in growth medium of *P. denitrificans*.

This type of in-line spectral analysis allows observation of metabolic processes in real-time for studies of various biological phenomena. Previously, we reported IR metabolomic data for *E. coli*¹². In industrial processes, in-line analysis allows optimization of growth conditions during the lifecycle of microorganisms. This is particularly useful, for example, during observation of fermentation processes in wine making, where IR spectroscopy can follow, quantitatively, the consumption of sugars and formation of alcohol. Another

example is the microbiological analysis of water, where the detection of CO₂ in fermentation processes by this infrared method¹⁷ can provide an early alert related to the presence of total coliform and *E. coli*¹⁸. Certain metabolomic patterns can be indicative of pathogenic bacteria in biofluids can be implemented in medical diagnostics¹². This example demonstrates that with appropriate engineering of methodology, the applications are unlimited. The next section discusses practical considerations for IR optical components and IR signal stability for application in bioreactors.

IV. Transition from FTIR to QCL platform: consideration of source, detectors and experimental conditions

While FTIR spectrometers are a robust tool in laboratory conditions, tunable QCLs have the advantages of being lightweight, small, rugged, modular instruments that can be used under a variety of environmental or field conditions.

We have previously reported data obtained from nitrate solutions in water using broadband Fabry-Perot (FP) QCLs¹¹. These broadband QCLs offer high stability and can be used in non-dispersive types of instruments for fluid analysis.

For *spectral* analysis, spectrometers based on tunable QCLs are commercially available. However, the transfer of measurements from FTIR to tunable QCL spectrometers can be challenging. In fluid analysis – such as field-grade wastewater analysis or bioreactor applications, one of the critical requirements is the stability of the infrared signal during the time interval between the measurement of a reference sample (for example, clean water) and measurement of a sample with contaminants or with metabolites. In a simple case of in-line sampling the time of the measurements *plus* the time for replacement between the reference and the actual sample takes no longer than 2 to 5 minutes. During this time, the infrared signal must stay stable. If an infrared spectral signal experiences any fluctuations, the sensitivity will decrease. In the following we will present the aspects of FTIR that allow acquisition of high-quality data under lab conditions and will discuss implications for transition of methodology to QCL-based systems.

It is not possible to directly compare FTIR and QCL spectra, because of the fundamental differences between the radiation sources, operational principles and method of detection. In FTIR, the global source is a thermal silicon carbide rod that is electrically heated for optimal emission of infrared radiation. The radiation is emitted continuously. The radiation from the source passes through an interferometer, and spectral conversion is achieved through Fourier Transform

algorithms. In our experiments described below, we used a Thermo Fisher FTIR spectrometer model IS-50, with a liquid-nitrogen cooled MCT-A detector.

In a tunable QCL-based spectrometer (LaserTune mini-QCL-200, 2 Tuner, spectral range $6\mu\text{m}$ - $10.5\mu\text{m}$ from Block Engineering), the QCL's external cavity¹⁹ is a Littrow configuration with back extraction²⁰. A grating element acts as a wavelength-tuning element. Depending on the angle between the incident radiation and the grating, a specific wavelength is selected. The selected wavelength couples back into the QCL chip. Unlike FTIR's continuous thermal source, the QCL operates in a pulsed mode, creating a further complication for comparisons.

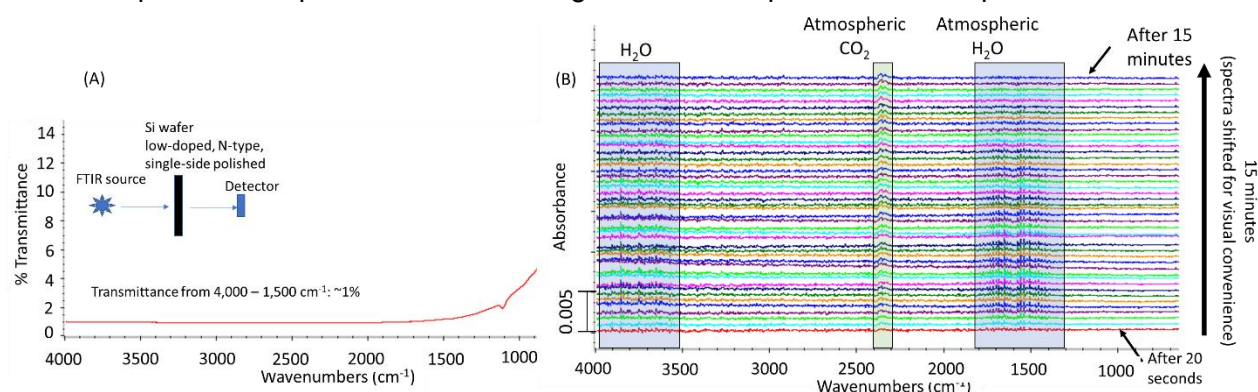


Figure 4 A. FTIR data: Optical properties of attenuator (low-doped Si wafer, N-type, single-side polished). (B) IR spectra obtained with the Si element in the beam pathway. The integration time was 20s. Each spectrum was referenced to a spectrum obtained at time $t=0$ s, for calculation of absorbance.

However, from a spectroscopist's perspective, the transition of fluid analysis from an FTIR to a tunable-QCL platform requires signal stability in terms of *signal-to-noise ratio* and *drift*. In our case, dealing with wastewater samples or with bioreactors, long-term stability over a time scale of 5 minutes is important. An example of typical stability of an FTIR signal is presented in Fig. 4. In Fig. 4(A) we provide optical parameters of an attenuator that we used in the beam pass (see

inset in Fig. 4A). This optical element was a piece of a single-side polished, low-doped, N-type Si wafer, with transmittance of roughly 1%.

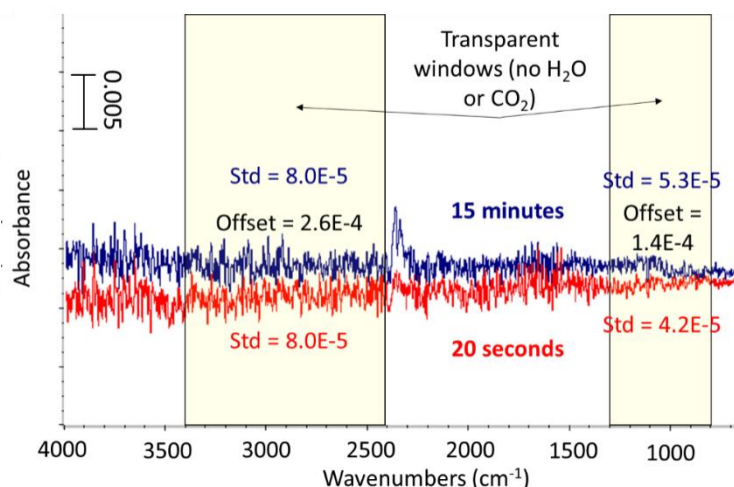


Figure 5 FTIR offset and standard deviation after 20 sec and after 15 minutes

Fig. 4B were shifted for visual convenience. The excellent spectral stability allows performance

of high-quality analysis of fluids, even when the time duration between the reference and the sample is on the scale of minutes.

Fig. 5 shows the detailed spectra after 20 sec. and after 15 min, (the first and the last spectra from Fig. 4B). Here, we show the data as-is, without application of any shifts. Both spectra were referenced to the same initial spectrum obtained at time $t=0$ s. The values of the drift (“offset”) and standard deviation of noise levels are indicated in Fig. 5 in the spectral ranges that are free of atmospheric absorptions due to water or CO_2 . There is practically no change in noise levels after 15 minutes, and the offset in absorbance is below 3×10^{-4} . This excellent stability allows acquisition of high-quality spectra in analysis of complex fluids. This stability is even more important in bioreactors where the processes are slow and the measurements are obtained hourly (such as in the data in Fig. 3).

Fig. 6A shows a general setup that we use for analysis of fluids using a tunable QCL spectrometer

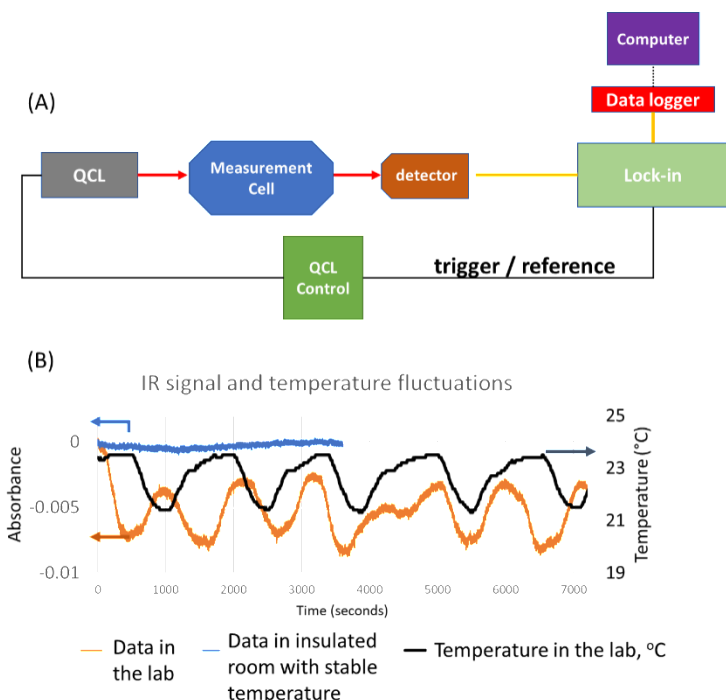


Figure 6. QCL setup for fluid analysis. The “measurement cell” is equipped with ATR waveguide and fluid (water). (B) Signal stability in the lab with uncontrolled and controlled environments. All data obtained at 1345cm^{-1} . Orange plot: IR signal in a standard lab; Black: Temperature fluctuations in the lab. Blue: IR signal in an insulated lab with stable temperature.

recorded in the lab is presented by a black curve, roughly correlating with the IR signal fluctuations. The temperature cycles are due to the operation of the air conditioning unit in the lab that controls the temperature between 21°C and 24°C . Moving the setup to a well-insulated lab with stabilized temperature condition removed the fluctuations (data shown by a blue line in Fig. 6B). The fluctuations observed in the QCL spectrometer/detector system in a laboratory with an air conditioning unit make the field-grade applications challenging. In addition, we found that the mechanical noise due to inclusion of mechanical fans to cool QCLs and the spectral continuity in

(LaserTune). A lock-in amplifier was used to collect the data, triggered on the QCL pulse. The measurement cell includes a waveguide and is filled with a fluid analyte (similar to the ISMIR cartridge in Fig. 1B). Selection of the detector is critical for the spectrometer. FTIR spectrometers usually come equipped with DTGS or liquid-nitrogen cooled MCT detectors. On the other hand, thermoelectrically cooled (TEC) MCT detectors tend to add signal fluctuations. Fig 6B shows data obtained using the LaserTune system with TEC-MCT detector from Block engineering (similar data was also obtained using TEC-MCT from Vigo, model PVMI-4TE-8-1x1-TO8, not shown here). In this case, the data was recorded at a constant wavelength (1345 cm^{-1}) with 300ns pulses at a $6\mu\text{s}$ period. Fig. 6B shows the IR signal as absorbance (the plot is shown in orange). All data was referenced to the first data point obtained at $t=0$ s. Temperature

the overlap between individual QCL chips are important factors when dealing with QCL-based spectrometers.

However, the above factors, once identified, can be addressed and resolved. Our experience with non-dispersive FP-QCLs indicates good potential for fluid analysis; these lasers can be manufactured in volume for specific spectroscopic bands and have high power output. Unlike gas analysis where the absorption bands are sharp, components in fluids result in broad absorption bands for which FP-QCLs are ideal. A system consisting of a FP-QCL, a detector and an ATR waveguide element for fluid analysis is small in size, and attractive for field applications or environments outside the laboratory

Beyond systems based on discrete components, epitaxial growth of QCLs and the subsequent material processing opens up various possibilities for photonic integrated circuits (PICs). The material structure of QCLs or interband cascade lasers (ICLs) can also support fabrication of quantum cascade detectors (QCDs) or interband cascade detectors (ICDs). There are multiple theoretical and experimental reports available on QCL/QCD devices^{21,22} and ICL/ICD²³ designs, which target gas analysis. For water analysis, these PIC platforms can incorporate IR waveguides for ATR sensing. Our future work will focus on design and application of miniaturized PIC platforms for analysis of fluids. Such devices will enable handheld applications for the field or integration with Unmanned Aerial Vehicles (UAV), where small weight and ruggedness are of high importance.

V. Summary

In summary, Max-IR Labs' ISMIR™ technology enables transfer of infrared analysis directly to wastewater treatment plants. This technology has multiple potential advantages over existing sensors of nitrogen-based contaminants in water that include the ability to detect and distinguish nitrogen contaminants in a complex wastewater matrix; speciation to nitrogen species (nitrate, nitrite and ammonia); low maintenance due to lack of a need for instrument calibration; and automated / autonomous operation.

Wastewater treatment plants are large-scale, complex bioreactors. At a lab-scale, Max-IR Labs is developing measurement methodologies that are compatible with either in-line or in-situ analysis of metabolic activities for microorganisms such as yeast and bacteria. In this manuscript we provide analysis of FTIR signals that present high spectral and temporal stability. This stability is critical for analysis of chemicals in wastewater or fluids in bioreactors, where reactions may take minutes or even hours. Transfer of experiments from FTIR to QCL-based setups therefore requires that the stability of the QCL / detector system match the time scale of the reactions of interest.

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