

Measures for optimizing pulsed EC-QC laser spectroscopy of liquids and application to multi-analyte blood analysis

M. Brandstetter, C. Koch, A. Genner and B. Lendl*
Vienna University of Technology, Getreidemarkt 9/164AC, Vienna, Austria

ABSTRACT

We employed a broadly tunable pulsed external cavity (EC)-QC laser with a spectral tuning range from 1030 cm⁻¹ to 1230 cm⁻¹ and a tuning speed of 166 cm⁻¹/s for direct absorption spectroscopy of aqueous solutions. The laser offered spectral power densities of up to four orders of magnitude higher than available with a conventional FTIR spectrometer. Therefore, a portable demonstration system with a large optical path length transmission flow cell (165 μm) could be realized preventing clogging of the flow cell. In pulsed mode an EC-QC laser provides significantly higher peak power levels than in continuous-wave mode, but pulse-to-pulse intensity variations, intra-pulse mode hops and mechanical imperfections of the scanning mechanism significantly impair the quality of resulting absorbance spectra. This article reports on measures which we found appropriate to reduce the initially high noise level of EC-QC laser absorbance spectra. These measures include a spectral self-referencing algorithm that makes use of the inherent structure of the EC-QC laser's gain curve to correct laser instabilities, as well as Fourier filtering, among others. This enabled us to derive infrared spectra which were finally useful for quantitative analysis in blood plasma samples. Finally, with the appropriate measures in place and using partial least squares regression analysis it was possible to simultaneously quantify 6 blood analytes from a single physical measurement of a 200 μL blood sample. This proves the potential of EC-QC lasers for practical application in clinical point of care analysis.

Keywords: Quantum cascade laser, External cavity, Pulsed operation, Liquid, Clinical diagnostics, Blood, Point of Care

1. INTRODUCTION

A direct absorption approach for measuring aqueous samples is particularly attractive as it involves only minimum optical requirements and the sample-light interaction can be realized in a flow-through or stopped-flow configuration enabling automation and the potential for further sample analysis, e.g. by other, orthogonal methods. Mid-IR spectroscopy offers the advantages of molecular specific, fundamental absorption bands, making simultaneous multi-analyte detection possible. Water, the predominant solvent in biochemical systems and of biological samples, shows large absorption in specific regions of the mid-IR range and thus dictate the design of possible measurement configurations.

The gold standard for analyses in the mid-IR region is Fourier transform infrared (FTIR) spectroscopy, where thermal emitters are used as infrared light source and, usually, Michelson-Morley interferometers are employed. A wide range of spectrometers from high-resolution bench-top instruments to smaller, robust, lower-resolution process spectrometers are commercially available. Aqueous samples, however, pose a challenge, since the limited emission power of thermal emitters makes short interaction path lengths necessary (depending on the spectral range of interest between 10-50 μm). These short path lengths impair system robustness especially when investigating biological samples because of cell clogging. Furthermore, sensitivity is limited as Beer-Lambert law is obeyed which states that the absorption A is proportional to sample concentration c , interaction length d and the decadic molar absorption coefficient ϵ :

$$A = -\log\left(\frac{I}{I_0}\right) = \epsilon \cdot c \cdot d \quad (1)$$

*bernhard.lendl@tuwien.ac.at; phone 43 1 58801-15140; fax 43 1 58801-15199; cta.tuwien.ac.at/epa/pa

Quantum cascade (QC) lasers, which have been available for over a decade now, offer a number of properties making them particularly interesting for mid-IR spectroscopy: high optical power and, in specific configurations, large tunability, e.g. external-cavity (EC)-QC lasers. These EC-QC lasers are so far the only commercially available tunable QC lasers, arrays of single mode distributed-feedback QC lasers are currently under development. Fig. 1a shows a comparison of the spectral emission power of a thermal emitter as used in FTIR spectrometers and a commercial EC-QC laser. The optical power per wavenumber (=spectral power density) is typically 3 to 4 orders of magnitude higher for set-ups based on EC-QC lasers than the spectral power density available inside the sample compartment of a FTIR spectrometer employing a thermal emitter. The need for high power mid-IR sources becomes clear when considering the low radiation power that is transmitted through a 50 μm water layer by a thermal emitter (blue spectrum in Fig. 1a), especially in the region of interest for blood (parameter) analysis. Thus it may be expected that the advantages of the EC-QC laser in terms of optical power would directly translate into improved performance when compared to FTIR systems equipped with a thermal light source.

When judging the quality of an analytical system two different aspects have to be considered. The first aspect involves metrological properties which can be judged using parameters such as accuracy (agreement of “true” value with the result of analysis) and precision of measurement (described by the standard deviation of the result of repeated analysis), among others. In addition to this metrological aspect there are parameters with a practical connotation like time and cost of analysis, mechanical robustness and simplicity of measurement. These parameters contribute to the second, practical aspect which is equally important to judge the quality of an analytical system. Both aspects need to be considered when it comes to evaluate whether a given technique is “fit for purpose”, i.e. whether it is able to solve a given analytical-chemical problem.

In IR spectroscopy two simple tests are often run to judge the metrological quality of a given experimental set-up for absorbance measurements: the 100%-line and the S/N ratio. Considering a transmission measurement, as dealt with in this paper, a practical parameters of relevance would be robustness of the measurement system which will mostly be governed by the risk of clogging the narrow flow cell.

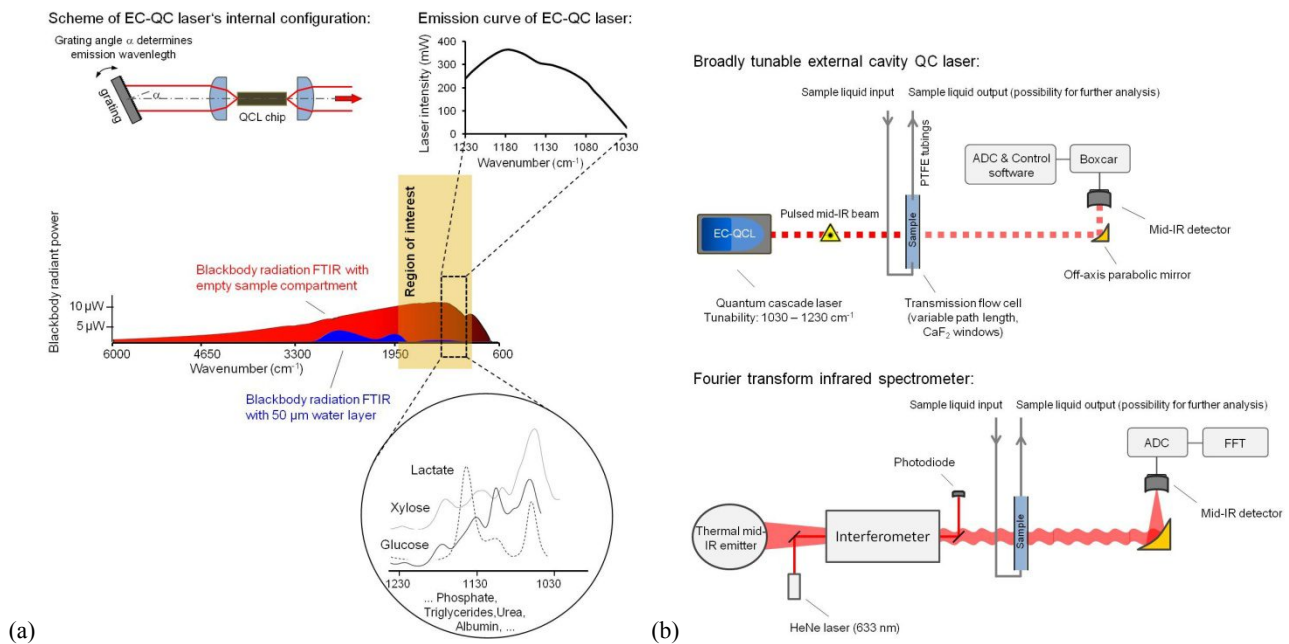


Figure 1. (a) Emission characteristics of thermal light source employed in the FTIR instrument (red spectrum) and EC QC laser (solid, black line). The blue spectrum shows the remaining optical intensity after placing a 50 μm water layer in the sample path, illustrating the need for a more powerful mid-IR source. (b) Direct absorption measurement schemes typical for liquid phase measurements: (bottom) FTIR instrument with a thermal light source (global). The dispersive element in this configuration is the interferometer; (top) mid-IR laser based set-up with a broadly tunable EC-QC laser which is at the same time the dispersive element.

100%-line and signal to noise ratio: A 100% line is the result of recording two single beam spectra without changing the sample and it is calculated as the absorbance spectrum of these two single beam spectra according to eq. 1. When analyzing aqueous solution in transmission, the corresponding 100% line is usually recorded with the flow-cell filled with distilled water. In case of ideally noise free measurements, a straight line at 0 absorbance would be obtained. However, in a real world set-up there will always be random fluctuations resulting in a characteristic noise level of the 100%-line, which is often reported as the residual standard deviation or the peak-to-peak noise. In case of absorbance measurements the “signal” of an analyte often refers to the magnitude of a characteristic band in the absorbance spectrum, which is measured under the same condition as the 100%-line; e.g. a glucose solution recorded against distilled water. The metrological quality of an absorbance spectrum of a sample will thus be determined by both, the amplitude of the absorbance spectrum as well as the noise level in the spectral area of interest.

2. MATERIALS AND METHODS

All measurements were performed in direct absorption configuration, realized as single-channel set-ups, i.e. no reference detector or reference analyte was involved (Fig. 1b). This configuration is typical for FTIR instruments and thus particularly suitable for the evaluation of the herein investigated broadband EC-QC laser in comparison to FTIR spectroscopy, the gold standard in mid-IR spectroscopy. The basic difference between the two methods is the non-dispersive nature of FTIR measurements, whereas EC-QC lasers are inherently dispersive due to spectral broadband tuning. Mid-IR detectors typically require signal modulation for maximum sensitivity. In a FTIR approach modulation of the continuous beam emitted by a thermal light source is achieved by the moving mirror of the interferometer. In case of the EC-QC laser this can for instance be realized by pulsed operation. Hence, no additional mechanical parts are involved which could increase vulnerability to mechanical shocks or vibrations. Table 1 summarizes some key numbers and basic differences of QC laser-based and FTIR (=thermal emitter-based) spectroscopy.

Table 1. Key numbers of FTIR and EC-QC laser based spectroscopic set-ups. The latter is focusing on QC lasers for liquid phase analysis, i.e. broadband, high power and pulsed operation.

	FTIR	EC-QC laser
Optical dispersion	Fourier-transform	dispersive
Available optical power (at the sample)	30-40 mW ¹	typically > 100 mW
Spectral power density (per cm ⁻¹)	< 15 μW ¹	
Spectral coverage	> 4000 cm ⁻¹ (full mid-IR)	up to 676 cm ⁻¹ ²
Maximum heat transfer to sample ^x (60 s measurement time)	~ 2.1 J (~ total optical power 35 mW)	1.5 J (5% duty cycle, 500 mW peak pulse power)
Signal modulation	mechanically by moving interferometer mirror	electrically, e.g. pulsed mode operation
Commercial availability	since 1960's	since 2008
Technological maturity / room for improvement	high / low	medium / high

^x... assuming the worst case scenario that all incident light is absorbed by the sample

EC-QC laser based absorption set-up: The EC-QC laser used in this study was a pulsed laser with a total tuning range from 1024.9 cm⁻¹ to 1230 cm⁻¹ (see Fig. 1a), a maximum repetition rate of 100 kHz and 500 ns maximum pulse length (Daylight Solutions, purchased 04/2009). Standard operation of the laser consisted of quasi-simultaneous spectral broadband tuning over the whole emission range utilizing the external grating of the EC-QC laser. Time-resolved characterization of laser pulses and spectral scans was reported earlier³. A full spectral scan required 1.2 s, i.e. a total number of 120k pulses reached the sample at maximum repetition rate. Details on data analysis are discussed in

section 4. The employed analog-digital converter (ADC) was a 24 bit AD7760 (Analog Devices). A thermoelectrically (TE) cooled Mercury-Cadmium-Telluride (MCT) detector (Infrared Associates) with a detectivity D^* of $4 \times 10^9 \text{ cm Hz}^{0.5} \text{ W}^{-1}$ served as detector for transmitted mid-IR radiation. Light-sample interaction took place in a transmission flow cell. The cell consisted of an aluminum body and two 2 mm CaF_2 windows separated by polytetrafluoroethylene spacers ($135 \mu\text{m}$ for standard solutions and $165 \mu\text{m}$ for clinical application). The cell volume was approximately $20 \mu\text{L}$. Absorption spectra were calculated from an initially acquired background spectrum (I_0) of Ringer solution (blood measurements) or pure water (all other measurements) and subsequently acquired sample spectra (I).

The reference FTIR spectrometer used in this study was a Vertex 80v (Bruker Optics) with a maximum spectral resolution of 0.07 cm^{-1} .

Multivariate data analysis: Multivariate analyses of acquired blood spectra were based on a Partial least squares (PLS) regression analysis using the PLS Toolbox (Eigenvector Research) for Matlab (Mathworks). Validation of results was based on the random subset method (10 data splits, 5 iterations).

Blood samples: Blood plasma samples were obtained as aliquots of routinely taken samples from intensive care unit patients at Vienna General Hospital. Details on sample acquisition and sample handling can be found in⁴. Reference analysis required for establishing multivariate calibration models were obtained from the hospital's clinical laboratory. The sample set consisted of a total number of 67 specimens. $200 \mu\text{L}$ of sample volume were required for a single measurement.

3. ANALYTE SIGNAL IN TRANSMISSION SPECTROSCOPY

The substantially higher optical power levels of QC lasers enable larger optical path lengths as compared to FTIR instruments. The path length is not only important regarding the robustness of the set-up but, furthermore, in terms of absorption signal which is linearly depending on the path length d . The level of improvement can be calculated from Beer-Lambert law (Eq. 1). We want to give an estimation of the achievable improvement when increasing the source intensity ($I_{FTIR} = I_0$) by a certain factor (k_I) by application of a QC laser with intensity I_{QCL} . This evaluation is based on the assumption of a certain minimum required intensity I_D incident on the detector, thus concentrating on the source influence, unaffected whether the measurement is dispersive or non-dispersive. In this case the maximum achievable optical path length d_{max} for a certain wavelength λ calculates as

$$d_{max,QCL} = \frac{1}{\epsilon c} \cdot \left(\log \frac{I_{FTIR}}{I_D} + \log k_I \right) \quad \text{with} \quad I_{QCL} = k_I \cdot I_{FTIR} \quad (2)$$

The product of molar absorption coefficient ϵ and molarity (or concentration) c can be replaced by the linear decadic absorption coefficient α , which is defined as the quotient of the decadic absorbance A and path length d .

Example: According to Eq. 2 the maximum path length consists of two terms: the basic path length achievable with the FTIR spectrometer and an additional term due to the increased source power of the QC laser. Assuming typical power levels for the laser (100 mW) and the thermal emitter of a FTIR spectrometer ($10 \mu\text{W}$ - according to¹; for $\sim 1 \text{ cm}^{-1}$ spectral range, to be comparable to the QC laser) the power enhancement factor results in $k_I = 10^4$. The maximum achievable path length is then:

$$d_{max,QCL} = \frac{1}{\alpha} \cdot \left(\log \frac{I_{FTIR}}{I_D} + \log k_I \right) = d_{max,FTIR} + \frac{1}{\alpha} \cdot 4 \quad \text{with} \quad d_{max,FTIR} = \frac{1}{\alpha} \cdot \log \frac{I_{FTIR}}{I_D} \quad (3)$$

If we now assume pure water as sample substance with a molarity of 55.5 mol/L and a molar absorption coefficient $\epsilon = 4.449 \text{ L/mol} \cdot \text{cm}^{-1}$ at 1080 cm^{-1} (carbohydrate region) and, furthermore, a minimum required intensity reaching the detector of $I_D = 100 \text{ nW}$ (corresponding to an absorbance of 2), the maximum achievable optical path length $d_{max,QCL}$ results in:

$$\text{Water at } 1080 \text{ cm}^{-1}: \quad d_{max} = 40.5 \mu\text{m} \cdot 2 + 40.5 \mu\text{m} \cdot 4 = 243 \mu\text{m} \quad (4)$$

The value $40.5 \mu\text{m}$ is solely determined by the linear decadic absorption coefficient α . When switching to other spectral regions α can change significantly.

Given the increased path length, a significant improvement of experiments in terms of sample handling and vulnerability to clogging can be expected. Nevertheless, the above derivation clearly indicates that the increase in path length due to increased optical power of QC lasers is attenuated by the logarithmic behavior of a transmission measurement. This is in contrast to other techniques, such as photoacoustic spectroscopy where the signal is proportional to source intensity,⁶ thus leading to much higher signal enhancement.

4. DIFFICULTIES ENCOUNTERED AND POSSIBLE MITIGATION WHEN USING EC-QC LASER IN TRANSMISSION SPECTROSCOPY

Different sources of noise contribute to the overall performance. While not going into detail regarding the FTIR instrument (here we refer the reader to excellent previous publications and book chapters⁷) some EC-QC laser specific effects shall be discussed in the following.

4.1 Influence of mechanical grating during broadband scanning

Consecutive spectral scans achieved by quasi-continuous rotation of the EC grating do not always exactly match (Fig. 2a). There are various reasons for this spectral mismatch, for instance mechanical imperfections as well as triggering issues. Despite the fact that this mismatch was found to be small ($\Delta\nu \sim 0.3 \text{ cm}^{-1}$) it can result in large fluctuations in the corresponding absorption spectrum. The sample data shown in Fig. 2b obtained with a 135 μm path length and pure water reveal fluctuations of the absorption signal of up to 50 mAU where one would expect smooth absorption bands.

Spectral alignment of scans: Typically, a number of scans is averaged to reduce spectral noise. However, since the observed fluctuations are not random this approach is sub-optimal. A closer look on a broadband spectral scan reveals that the actual cause for these large fluctuations is the fine structure of the emission curve.⁸ A significant reduction of these fluctuations in the absorbance spectra can be achieved by a spectral alignment procedure which involves shifting of recorded scans to achieve maximum alignment (see Fig. 2). Time-consuming averaging steps can thus be avoided. The alignment procedure, however, does not consider the absolute spectral position. The maximum spectral mismatch observed during our measurements was in the range of $\Delta\nu = 0.3 \text{ cm}^{-1}$ which is low compared to typical absorption band widths of tens of wavenumbers in liquids. Nevertheless, in some critical cases, the absolute position of absorption bands might be required which calls for an additional measure (see section 4.2).

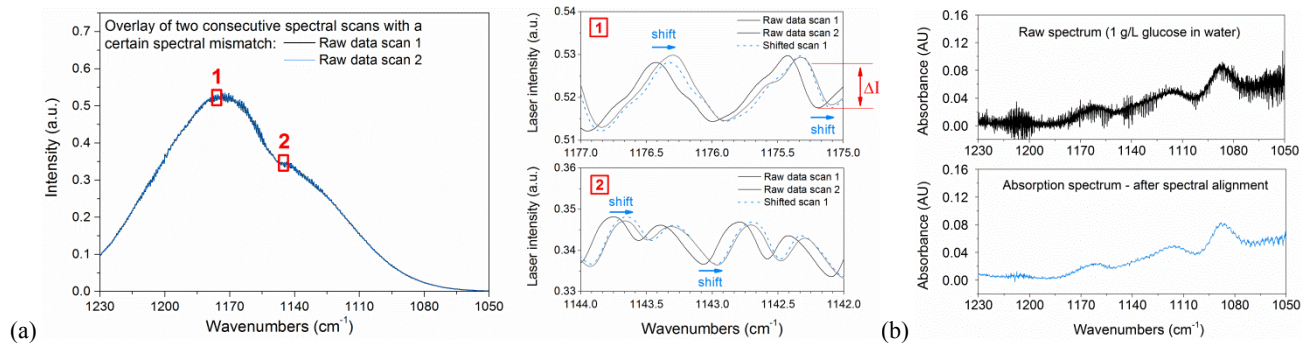


Figure 2. (a) Detector signals (=single beam spectra) during spectral tuning acquired with the set-up shown in Fig. 1 with a 135 μm water layer for attenuation of laser intensity. Insets 1 and 2 show enlarged sections of single beam spectra revealing its fine structure caused by laser mode-hops. Curves have been smoothed for better visibility. A mismatch between consecutive scans can be corrected by a spectral shift, the intensity scale remains unaltered. (b) Fluctuations resulting from spectral mismatch of scans (top) and improvement by spectral alignment (bottom) on the example of a 1 g/L aqueous glucose solution against a pure water background.

Fourier filtering: The absorption spectrum of glucose, shown in Fig. 2b (bottom) still exhibited low amplitude fluctuations after spectral alignment was performed. Further reduction of these fluctuations may be required to achieve

optimum spectral quality for subsequent data processing, such as multivariate data analysis. The straight-forward method would be averaging of scans, however, this does not result in sufficient improvement within a reasonable time frame, as acquisition of a single scan takes 1.2 s. Including backward tuning this increases to approximately 4 s. Furthermore, as already mentioned above, the fluctuations are not random noise which makes averaging ineffective. Hence, alternative filter algorithms are required, such as Savitzky-Golay filtering or Fourier-filtering. Fig. 3 illustrates achieved signal quality improvement by application of solely averaging (20 scans), Savitzky-Golay filtering and Fourier filtering on the example of an aqueous xylose solution spectrum.

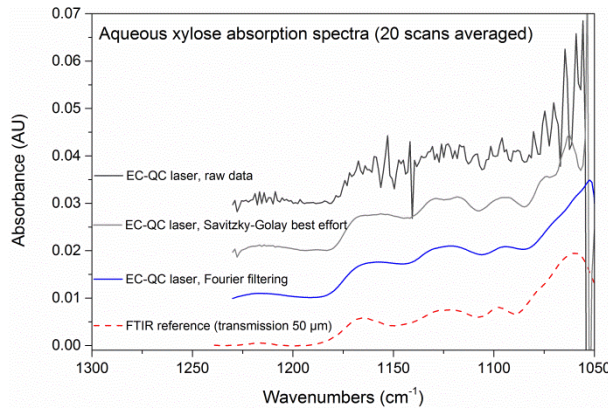


Figure 3. Effect of different filtering algorithms on spectral quality of recorded absorption spectra with EC-QC laser. A 0.75 g/L aqueous xylose solution was measured with pure water as background. The optical path length for the laser-based measurements was 135 μm . The reference FTIR spectrum was scaled by a factor 1.5 for better visibility.

Savitzky-Golay filtering (best effort, i.e. largest possible window size without decrease of absorption bands) was not capable of removing all fluctuations. Better results were obtained by application of a Fourier filtering algorithm (Fast Fourier transformation-FFT) of the obtained single beam spectra I and I_0 , performed in LabView (National Instruments, USA). Prior to Fourier transformation, a 3-term Blackman-Harris apodization function was applied to the spectra. In the Fourier-domain the spectra were shifted to achieve a center-burst like structure, similar to the interferogram of a broadband thermal emitter in a FTIR instrument. Subsequently, Blackman-Harris apodization was applied again. The window width was now adjusted to result in best possible reduction of fluctuations while at the same time maintaining analyte-specific absorption bands. The optimum width depended on the investigated spectral range and sample type. An example is given in for blood plasma samples (see section 5). Finally, an inverse Fourier-transformation was performed, resulting in smooth intensity spectra with almost no observable fluctuations. The absorption spectra shown in Fig. 3 prove the quality of the EC-QC laser spectra (135 μm path length flow cell) by comparison with reference FTIR spectra obtained with a 50 μm path length flow cell.

4.2 Wavelength reference

Basic wavelength reference: The basic spectral calibration of a QC laser can be achieved by determining the spectral information at each point in time during a quasi-continuous scan by a time-resolved FTIR measurement. This kind of calibration of the wavenumber axis with tuning time was presented earlier in ³. However, this reference does not provide maximum possible resolution, thus, in particular critical cases where maximum spectral accuracy is required additional measures have to be taken.

Improved wavelength reference: FTIR spectrometers benefit from an absolute wavelength reference obtained by a HeNe-laser which is aligned with the mid-IR probe beam of the thermal emitter. The HeNe-laser interferogram has the sinusoidal shape typical for single-mode sources which is then used as absolute wavelength reference to correct potential mechanical instabilities of the moving mirrors. Similar concepts have also been applied for QC laser based systems, mainly for gas phase spectroscopy. Most of these concepts rely on etalons or dual channel configurations with a reference substance with defined absorption bands. Some of these measures have drawbacks. The etalon, for instance is

ideal for highly resolved gas spectra, but not for liquid samples with broad absorption bands in the tens of wavenumbers regime. Similar considerations apply for reference channels, as absolute wavelength standards only have a certain number of spectral features available, therefore often limiting applicability for broadband spectra.

An attractive alternative inherent to EC-QC lasers is to make use of their characteristic fine structure that is superimposed to their emission curve. In section 4.1 we discussed adverse effects due to this fine structure, whenever consecutive scans are not perfectly aligned. Here, we want to demonstrate how it can be used as absolute wavelength reference. This is achieved by recording high resolution FTIR spectra (0.1 cm^{-1}) of the EC-QC laser emission at fixed grating positions. In that way the FTIR spectrometer serves as absolute wavelength reference. At a defined grating position the EC-QC laser typically emits several modes which cover a spectral region of about 1 cm^{-1} . The single spectra (Fig. 4a, bottom) were acquired with a spectral offset of 1 cm^{-1} between individual spectra and covered the whole tuning range of the EC-QC laser. Subsequently, they were combined and their envelope (Fig. 4a, top) served as a “ruler” for wavelength assignment. During data acquisition potential wavelength shifts induced by a too large aperture in the FTIR instrument have to be considered for ultimate wavelength accuracy.⁷

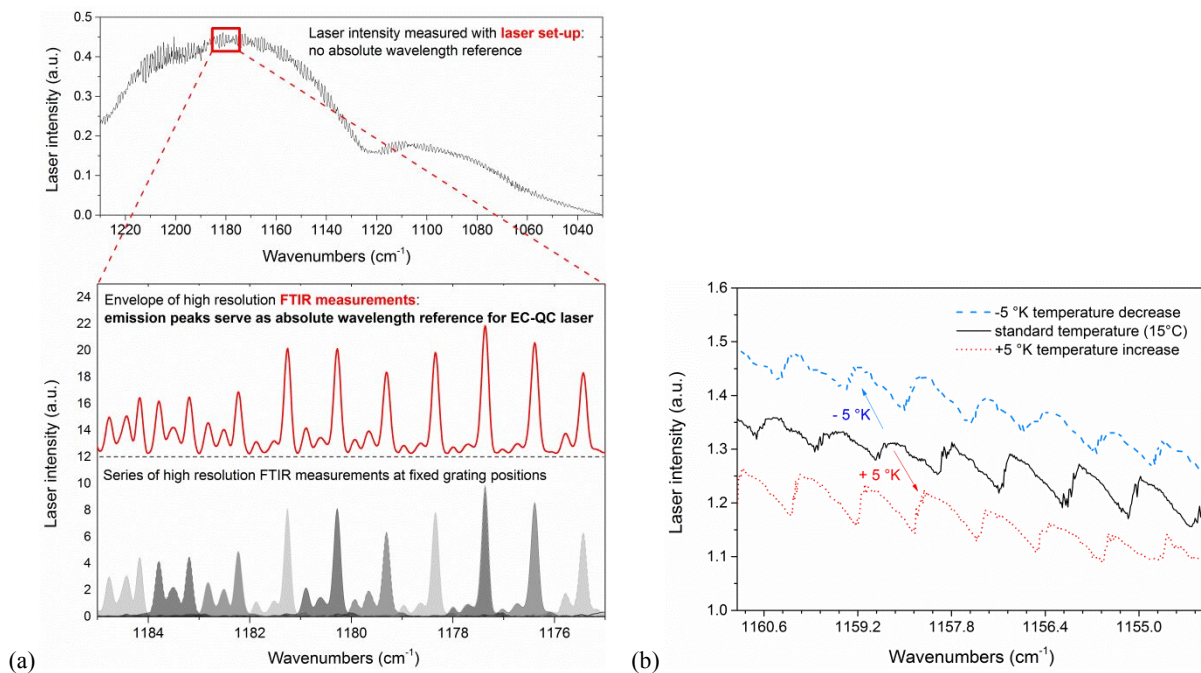


Figure 4. (a) Highly resolved emission spectra of EC-QC laser recorded with a FTIR spectrometer (0.1 cm^{-1} resolution, 1 mm aperture) at different positions of the EC-QC laser's grating. The highly structured emission characteristics caused by mode-hops is observed during spectral tuning. The spectral offset between single spectra as adjusted with the external cavity grating is 1 cm^{-1} . The envelope of single emission spectra can be used as absolute spectral reference for the laser by utilization of emission peak positions. (b) Influence of QC laser chip temperature on absolute position of characteristic fine structure.

The fine structure pattern is basically defined by the QC laser's chip and external cavity length and does not change as long as the operational parameters of the laser stay constant. The latter particularly addresses the laser chip temperature (here 15°C , according to the readout on the laser driver). If changed by the user, this would alter absolute position and shape of the gain-curve's fine structure as the laser chip mode-hops depend on the chip temperature. This effect is illustrated in Fig. 4b, where the laser chip temperature was varied over $10 \text{ }^\circ\text{K}$ which corresponded to a shift of approximately 1 cm^{-1} . Finally, it should be noted, that the referencing using high-resolution FTIR spectra is a one-time procedure which does not have to be repeated.

4.3 Pulse-to-pulse stability and data acquisition

With the term pulse-to-pulse stability we here refer to stability of emission power of subsequent pulses. Pulse jitter, i.e. deviations from pulse periodicity was not an issue during our studies since it was found to be well below 10 ns. Analog-digital conversion of the detector signal is normally performed with an ADC, which is suitable for low frequency modulated signal as, for instance, in a FTIR instrument. Direct conversion may, however, lead to limited performance in QC laser based set-ups as modulation rates are much higher here (typically 10 kHz-500 kHz). Since the sampling rate should at least be higher than the repetition rate an acceptable noise level may be challenging. Furthermore, in case of mode-hopping during a pulse significant fluctuations may occur if the digitalization point of the ADC is close to it. Lock-in detection cannot retrieve its full potential as duty cycles are typically <10%.

A suitable technique avoiding drawbacks of direct conversion and lock-in detection is boxcar integrated detection.⁹ Here, a boxcar shaped gate of adjustable length is triggered to overlap with desired portions of the pulses. Thus averaging of the laser pulse over a certain period is achieved. In our system we applied a modified boxcar integrator system with a sample/hold element instead of the gated integrator. This design results in reduced noise, less baseline drift and lower power consumption as compared to conventional designs (Fig. 5a, a detailed description can be found in ¹⁰). Furthermore, Fig. 5a shows a comparison of 100%-lines with pure water in a 135 μm optical path length transmission cell. Five lines were obtained by direct AD conversion with a triggered ADC (solid blue lines) and five lines were measured with boxcar-gated AD conversion (dashed black lines). The latter shows improved approximation to the ideal case of a flat line of 0 AU. Potential baseline drifts were corrected by dual-channel boxcar detection, where both, pulse and baseline, were measured and the difference used as detector signal.

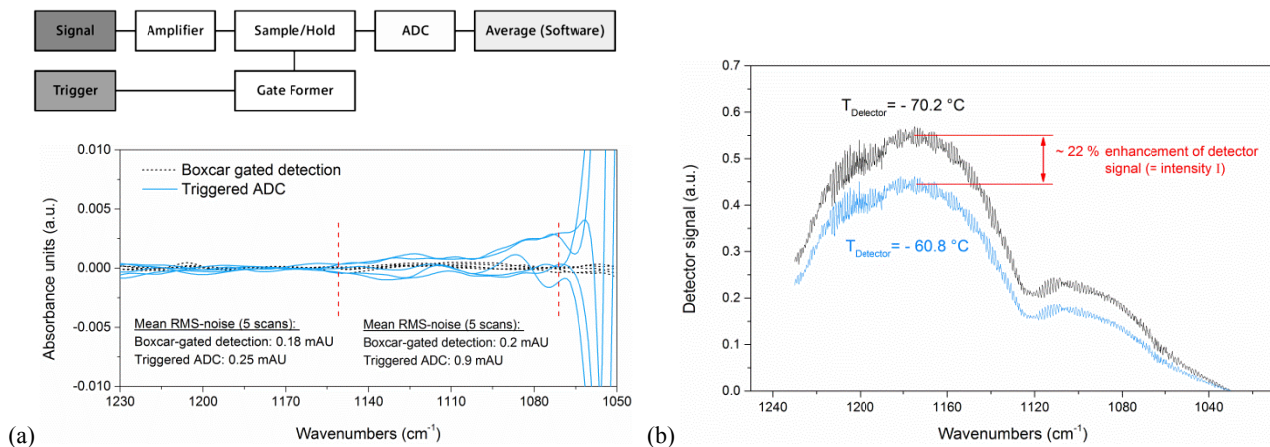


Figure 5. (a) Modified boxcar integrator for reduced noise, less zero drift and lower power consumption¹⁰, and 100%-lines recorded with different detection schemes: with triggered ADC detection and boxcar-gated detection suitable for pulsed laser emission. (b) Detector signals recorded during full spectral scan of the EC-QC laser at two different detector temperatures. The signal enhancement achieved by a 10 K detector temperature drop is approximately ~22%. Measurements were performed through a 135 μm water layer.

4.4 Detector temperature

Here we specifically refer to detector temperature control with thermoelectric cooling systems. The detector temperature is known to have significant influence on the detectivity D^* . Thus sufficient efforts must be taken to achieve a low and constant detector temperature. The measurement results, shown in Fig. 5b illustrate the temperature influence on the detector signal on the example of a 10 K detector temperature drop. While keeping source intensity I_0 constant, the detector signal I_D increased by approximately 22% when reducing the detector temperature from 60.8°C (blue curve) to -70.2°C (black curve).

5. APPLICATION TO CLINICAL BLOOD ANALYSIS

The performance of the laser based spectrometer shown in Fig. 1b (top) shall be demonstrated by results gained during a clinical study with details published earlier.⁴ For the study a portable demonstration unit was designed and assembled, including automated sample injection of up to eight blood plasma specimens. Spectra acquired with the demonstration unit were analyzed by multivariate PLS regression, thus enabling simultaneous quantification of several blood parameters. One of the main challenges in this application was the particular complexity of the sample set which cannot be reached by artificially prepared specimens.

Absolute wavelength referencing was not used for this specific application since the spectral accuracy obtained by basic wavelength referencing was sufficient. Five absorption spectra of each specimen were recorded and averaged. Prior to that, the resolution of raw spectra (92,000 data points) was reduced by averaging data point blocks of various sizes therein. Furthermore, Fourier filtering was applied. Differences in the size of apodization windows and spectra were corrected by appending the respective number of zero values at the end of the spectra. The optimum combination of spectral resolution and width of apodization window was determined by the calculation of the resulting root-mean-square errors of calibration (RMSEC) for each investigated analyte. Two possible combinations are summarized in Table 2 together with results of simultaneous quantification of 6 blood parameters in plasma samples. The effect of different filter widths on shape and position of blood plasma absorption bands is illustrated in Fig. 7 for two exemplary patients.

Table 2. Optimum Fourier filter configurations and results of simultaneous quantification of 6 blood analytes in plasma.

Determination of optimum Fourier filter width			
Nominal spectral resolution	Width of apodization window (Fourier filter)	No. of spectral data points	Impact on achieved calibration error (glucose) (RMSEC)
0.43 cm ⁻¹	512 data points	460	10.0 mg/dL (optimum)
0.87 cm ⁻¹	256 data points	230	11.4 mg/dL
Results of multivariate quantification of blood analytes in plasma specimens			
Analyte	Concentration range of specimen set	Achieved validation error (0.43 cm ⁻¹ / 512 points window width)	
Glucose	85 - 264 mg/dL	12.2 mg/dL	
Lactate	0.4 - 3.7 mmol/L	0.44 mmol/L	
Albumin	17.2 - 35 g/L	1.2 g/L	
Total protein	33.7 - 69.4 g/L	2.2 g/L	
Total cholesterol	36 - 225 mg/dL	20 mg/dL	
Triglycerides	45 - 266 mg/dL	21.5 mg/dL	

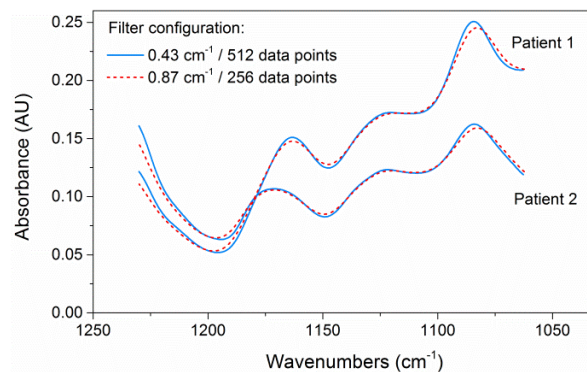


Figure 6. Absorption spectra of blood plasma specimens obtained from two exemplary patients. Solid and dashed lines indicate the influence of different combinations of spectral resolution and apodization window width: 512 data points (solid line), 256 data points (dashed line).

6. SUMMARY AND CONCLUSION

Pulsed operation EC-QC lasers are a powerful source for liquid phase analysis. Low duty cycles avoid unwanted heat transfer to the sample which would lead to sample alteration, while maintaining high peak power levels which allow for large optical path lengths. The latter can be increased by a factor of 3-5 and is limited by the logarithmic dependence of the possible path length on the available spectral power density. This increase in optical path length is often a decisive advantage in the analysis of real samples, such as human blood plasma due to the resulting significantly increased robustness of the overall experimental set-up as compared to FTIR based measurements. To meet the high quality of low noise measurements achievable by FTIR spectrometry, the obtained raw EC-QC laser spectra require substantial post-processing. In this paper we have discussed a number of strategies and measures for coping with different experimental challenges encountered during EC-QC laser experiments. Data post-processing can be of particular importance for subsequent data analysis steps, such as multivariate data analysis. A careful optimization of measurement parameters and data processing finally allowed reaching the metrological quality of EC-QC laser spectra required for the successful analysis of blood plasma samples. Here we report on the rapid simultaneous determination of six blood parameters out of 200 μL sample volumes. Recent results, not included in this paper, also show that considering blood plasma and serum, a total number of nine parameters can be determined simultaneously.

It should be noted, that the EC-QC laser employed for this study showed constant performance over a total period of 4.5 years. During this period, the laser system (including the driver) was operated for 3565 hours and the laser chip itself was emitting for 1454 hours, demonstrating the technological maturity of this first generation commercial EC-QC lasers. The technology of QC lasers is, however, still evolving and new, improved multi-wavelength devices may enter the market soon. This is why the analytical problem solving capabilities of analytical methods based on EC-QC lasers will most likely increase in the very near future. For the topic of point-of-care analyzers this will lead to small, light-weight instruments with the capability to simultaneously quantify nine parameters or even more and as such become an interesting alternative to established technologies.

ACKNOWLEDGEMENTS

The authors very much acknowledge the valuable support of W. Tomischko in all electronic-related issues. Moreover, we want to thank the team of the ICU 13H1 of the Medical University of Vienna. Financial support was provided by the Austrian research funding association (FFG) under the scope of the COMET program within the research network "Process Analytical Chemistry (PAC)" (contract # 825340).

REFERENCES

- [1] Brandstetter, M., Genner, A., Anic, K., and Lendl, B., "Tunable external cavity quantum cascade laser for the simultaneous determination of glucose and lactate in aqueous phase," *The Analyst* 135(12), 3260–3265 (2010).
- [2] Lehtinen, J., and Kuusela, T., "Broadly tunable quantum cascade laser in cantilever-enhanced photoacoustic infrared spectroscopy of solids," *Applied Physics B* (2013).
- [3] Brandstetter, M., and Lendl, B., "Tunable mid-infrared lasers in physical chemosensors towards the detection of physiologically relevant parameters in biofluids," *Sensors and Actuators B: Chemical* 170, 189–195 (2012).
- [4] Brandstetter, M., Sumalowitsch, T., Genner, A., Posch, A.E., Herwig, C., Drolz, A., Fuhrmann, V., Perkmann, T., and Lendl, B., "Reagent-free monitoring of multiple clinically relevant parameters in human blood plasma using a mid-infrared quantum cascade laser based sensor system," *The Analyst* 138(14), 4022–4028 (2013).
- [5] Bertie, J.E., and Lan, Z., "Infrared Intensities of Liquids XX: The Intensity of the OH Stretching Band of Liquid Water Revisited, and the Best Current Values of the Optical Constants of H₂O(l) at 25°C between 15,000 and 1 cm⁻¹," *Appl. Spectrosc.* 50(8), 1047–1057 (1996).
- [6] Tittel, F.K., and Lewicki, R., [Tunable mid-infrared laser absorption spectroscopy], in *Semicond. Lasers*, Woodhead Publishing Limited, 579–629 (2012).
- [7] Griffiths, P.R., and de Haseth, J.A., [Fourier Transform Infrared Spectrometry], Second ed, John Wiley & Sons, Inc, 560 (2007).

- [8] Wysocki, G., Curl, R.F., Tittel, F.K., Maulini, R., Bulliard, J.M., and Faist, J., "Widely tunable mode-hop free external cavity quantum cascade laser for high resolution spectroscopic applications," *Applied Physics B* 81(6), 769–777 (2005).
- [9] Hinkov, B., Yang, Q., Fuchs, F., Bronner, W., Köhler, K., and Wagner, J., "Time-resolved characterization of external-cavity quantum-cascade lasers," *Applied Physics Letters* 94(22), 221105 (2009).
- [10] Tomischko, W., and Lendl, B., "Ein vereinfachtes Boxcar-Integrator-System für Prozessanwendungen mit Gatesteuerung unter Verwendung von Standardbauelementen," in *Informationstagung Mikroelektron. IEEE Austria Sect.*, 204 (2012).