Quantum cascade laser spectroscopy in biomedical and forensic science

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Outline

- Introduction: mid-IR laser spectroscopic sensing
- Breath analysis: D/H isotope ratio measurement
- Surgical smoke analysis
- Non-invasive glucose sensing: QCL and PAS
- Cocaine detection in saliva: FTIR and QCL
- Conclusions and outlook

(Broadly) tunable narrow-band mid-IR lasers

Laser	Wavelength [μm]	Tuning Characteristics	Power	Operation
CO_2	9 – 11	Only line tunable	Watts	[illegible] operation
Sb-based interband cascade	2 – 5.3	Few nm	[illegible]0 mW	RT operation
QCL	4 – 12, THz	[illegible] > 100 [illegible] per device	mW to W	LN_2/TE cooling, also RT
DFG/OPO[a]	3 – 1[illegible]	~ μm for specific setup	μW to mW Watts	RT operation

Ideal laser source does not exist yet

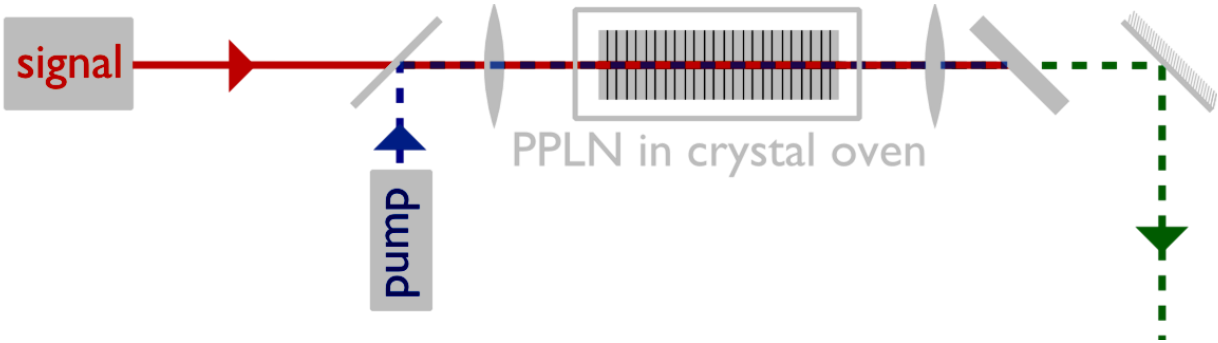
QCL: quantum cascade laser, RT: room temperature, LN_2: liquid nitrogen cooling, TE: thermoelectric cooling

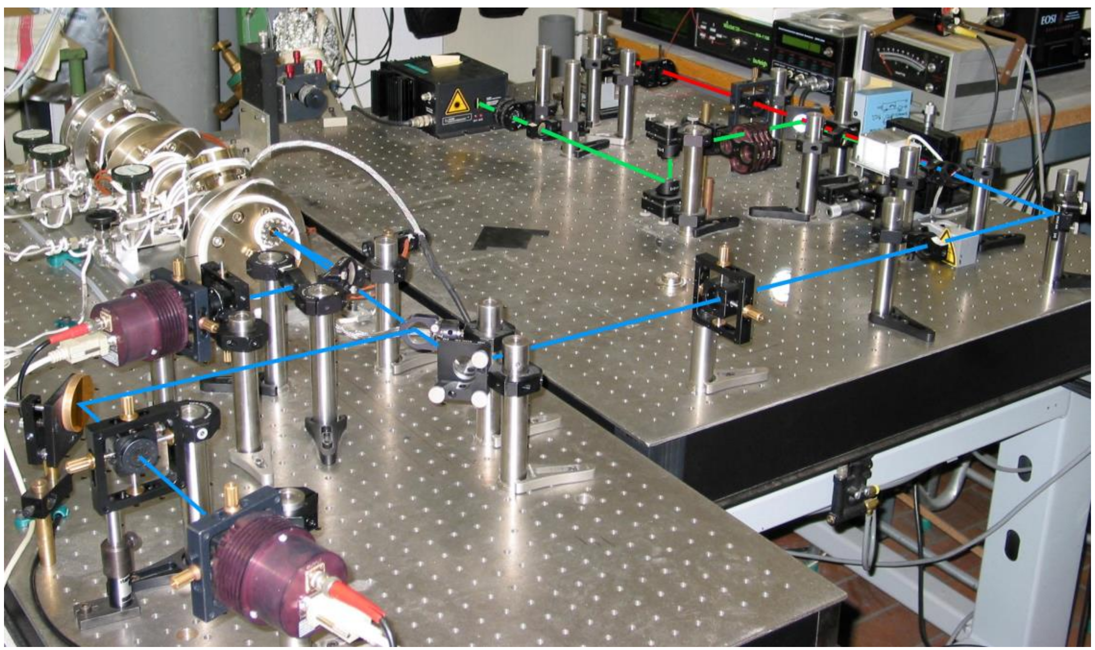
[a] DFG: difference frequency generation / OPO: optical parametric oscillator

Examples: PPLN (periodically poled lithium niobate, eventually with waveguide), $AgGaSe_2$, $LiInS_2$, $LiInSe_2$, etc.

Tunable narrowband mid-infrared lasers

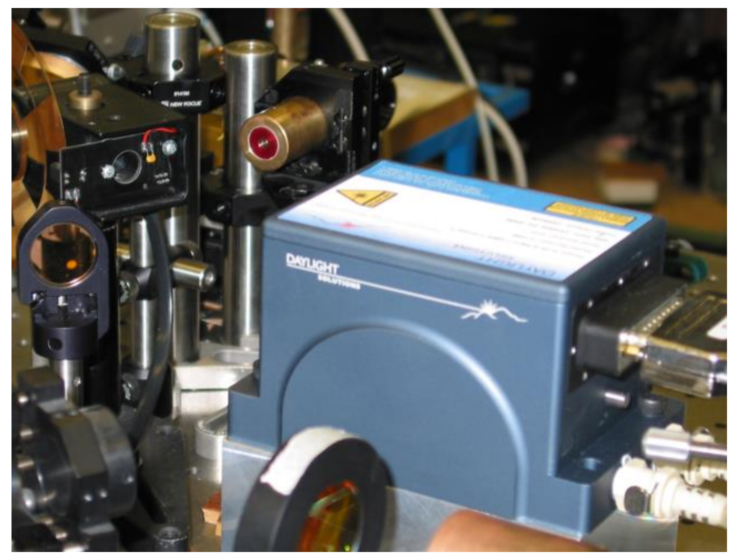
Difference frequency generation





RT, 3-17 μm, broad tuning

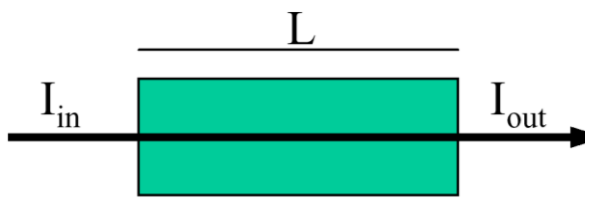
Quantum cascade laser



TE, 4-12 μm, “broad” tuning, compact

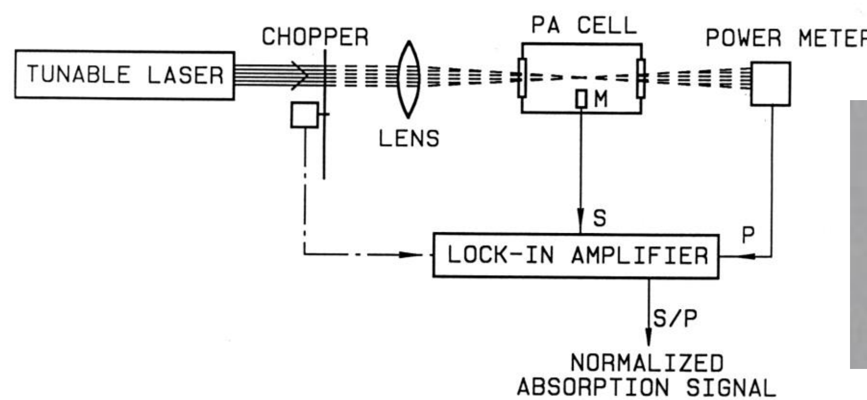
Absorption measurement: Detection schemes

Transmission



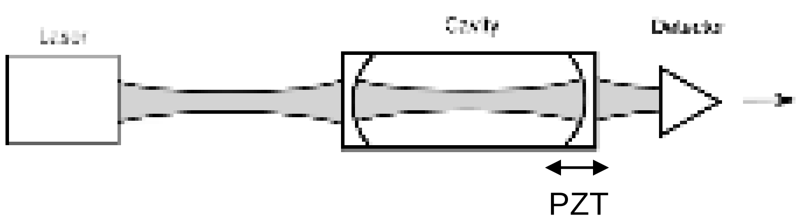
$$I_{out} = I_{in} \cdot \exp\{-\overbrace{N\,\sigma(\lambda)}^{\alpha(\lambda)}L\}$$

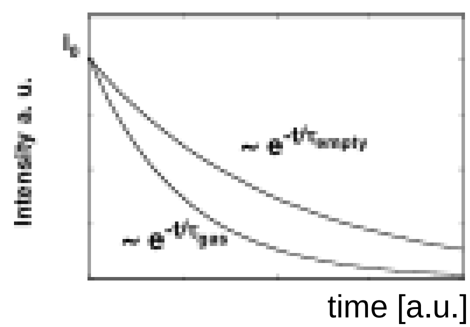
Photoacoustic



$$S(\lambda) = C\,P(\lambda)\,N_{tot}\,c_{gas}\,\sigma(\lambda)$$

Cavity Ring-down





$$\alpha_{gas} = \frac{1}{c}\left(\frac{1}{\tau_{gas}} - \frac{1}{\tau_{empty}}\right)$$

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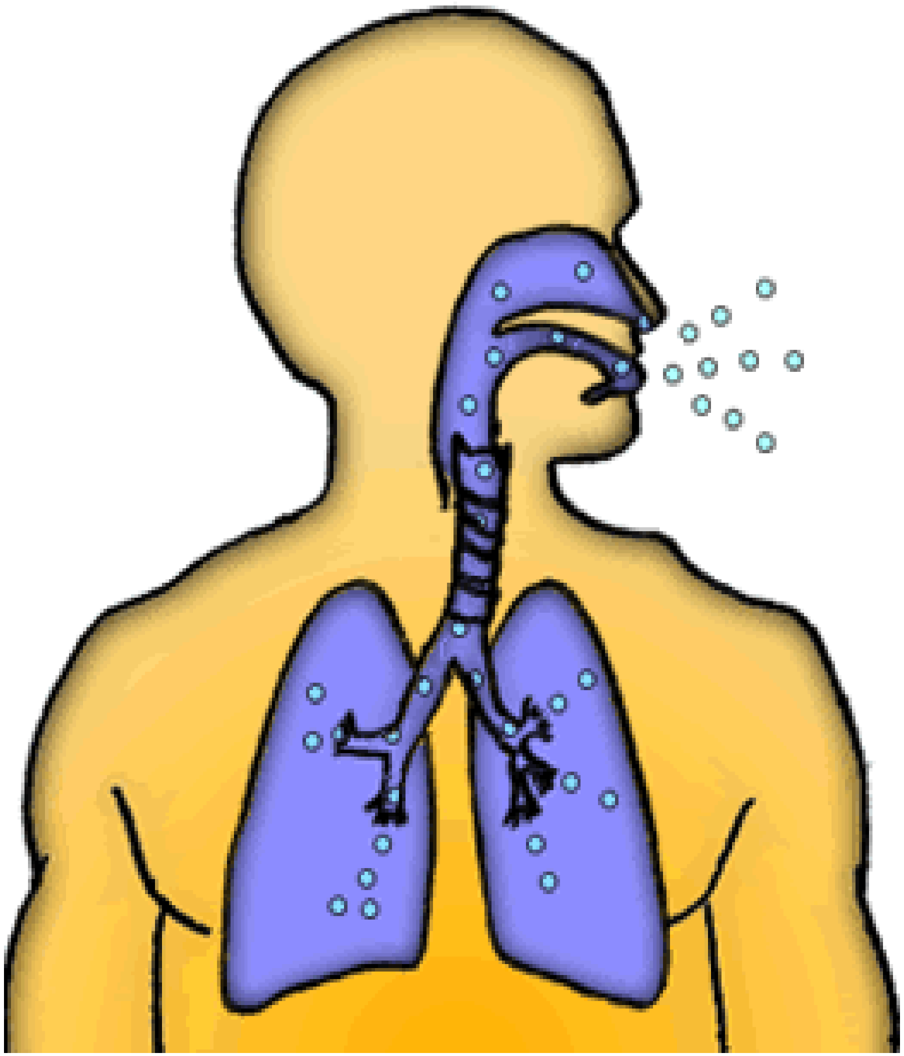
Human breath analysis



Concentration range	Molecule
% range	N_2, O_2, H_2O, CO_2
ppm range	CO, CH_4, NH_3, acetone
ppb range	NO, VOC's, …

Application type	Examples
Traditional monitoring	capnography
Biomarker tracing	NO (asthma), acetone (diabetes)
Stable isotope tracing	^{15}N, ^{13}C (H. pylori), ^{2}H

Deuterated tracers can be used to determine:

- Total body water
- Energy expenditure
- Glucose synthesis rates
- Cholesterol synthesis rates

Deuterated water (D_2O) as non-radioactive tracer

How to measure ?

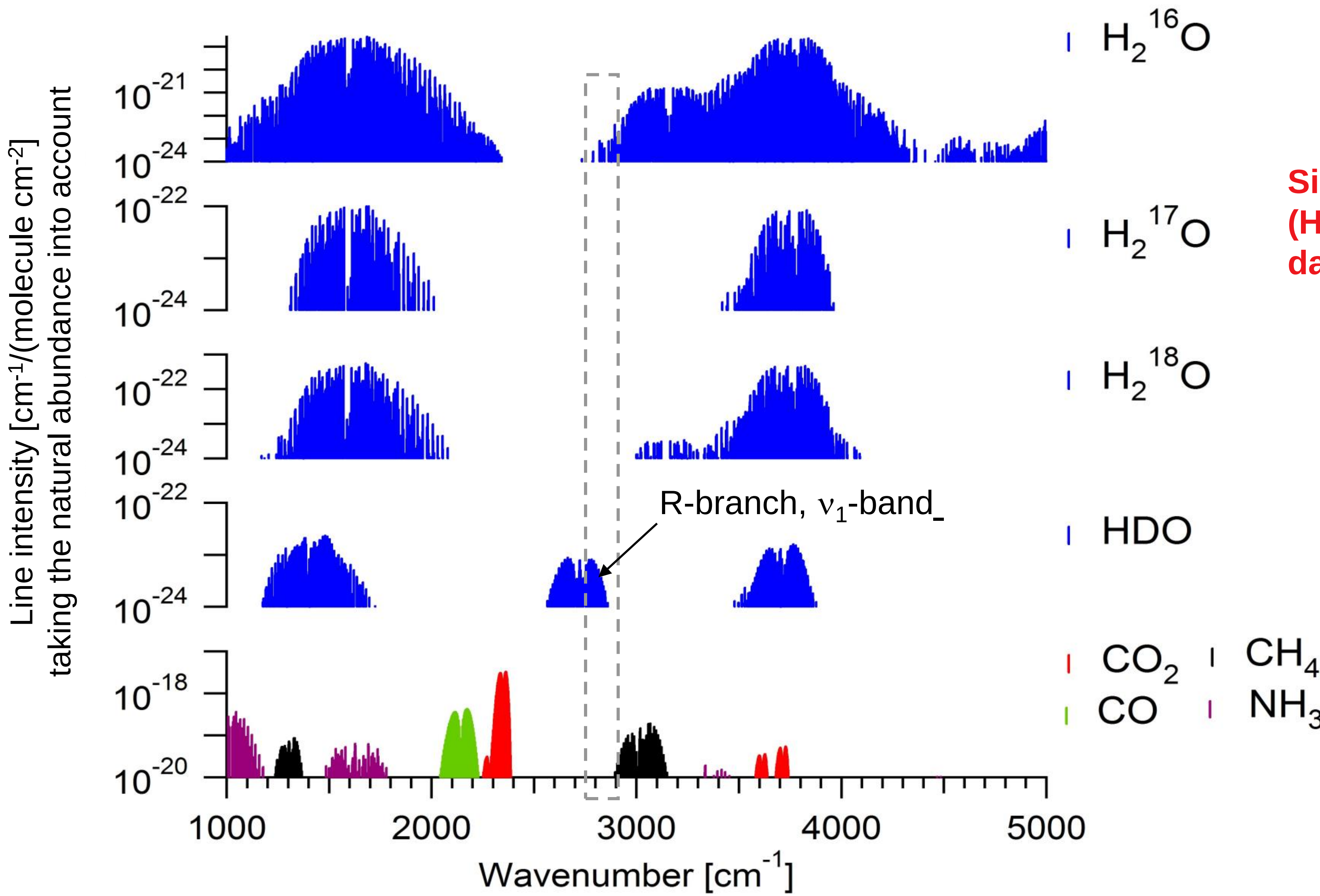
- Ingested D_2O mixes with water (H_2O) in the body
  → HDO

- HDO measurement in urine or saliva samples

Our approach:

Measure HDO directly in **breath** sample with laser spectroscopy

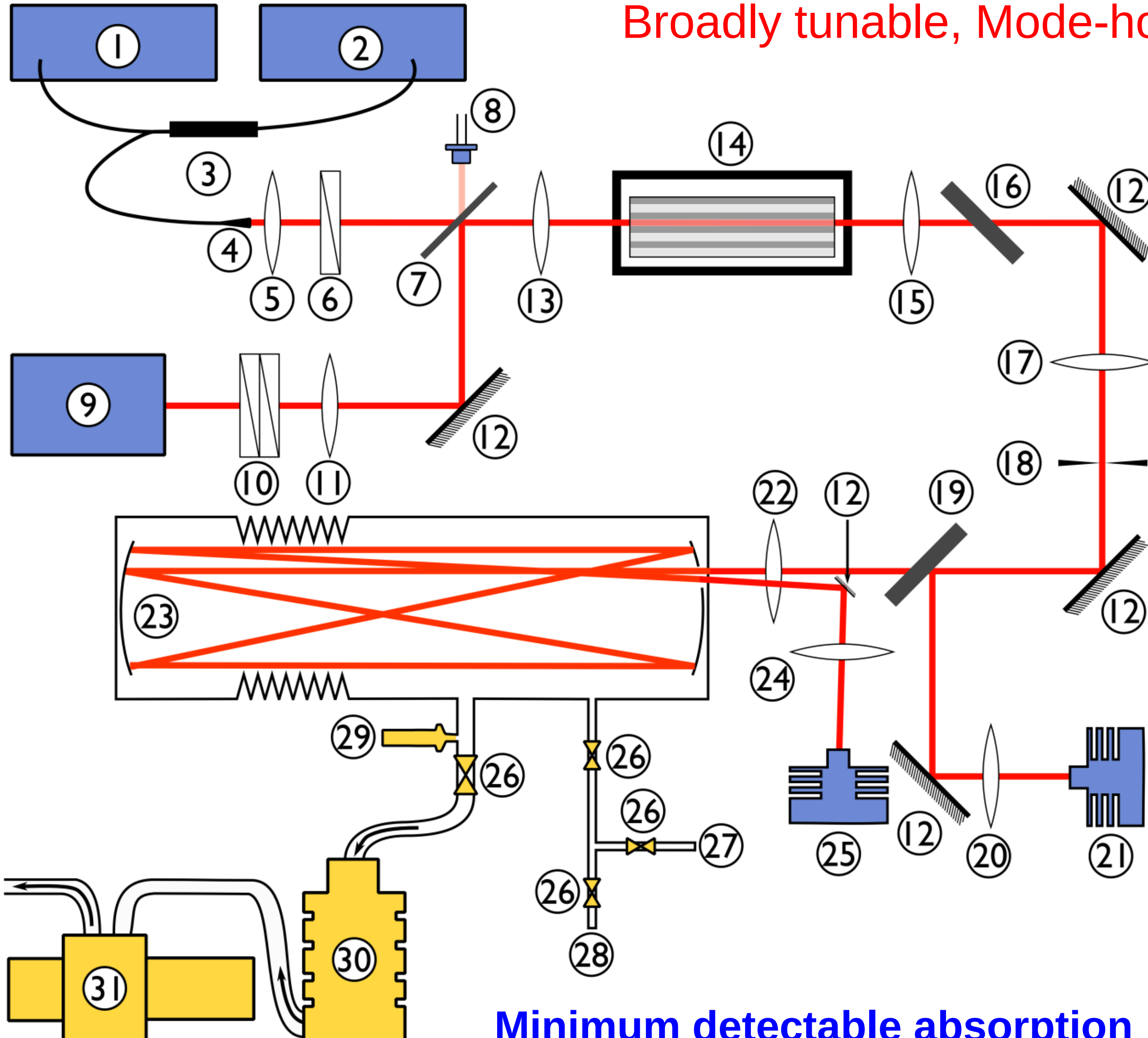
Analysis of human breath for biomarkers: D/H ratio



Simulation (Hitran database)

DFG spectrometer

Broadly tunable, Mode-hop free, Room temperature



1 ECDL, 1520-1600nm
5 mW CW

2 Wavemeter for ECDL

9 Nd:YAG, 1064.5 nm,
5 kHz, 6 ns, 300 mW av

14 PPLN, 5 cm, 8 periods

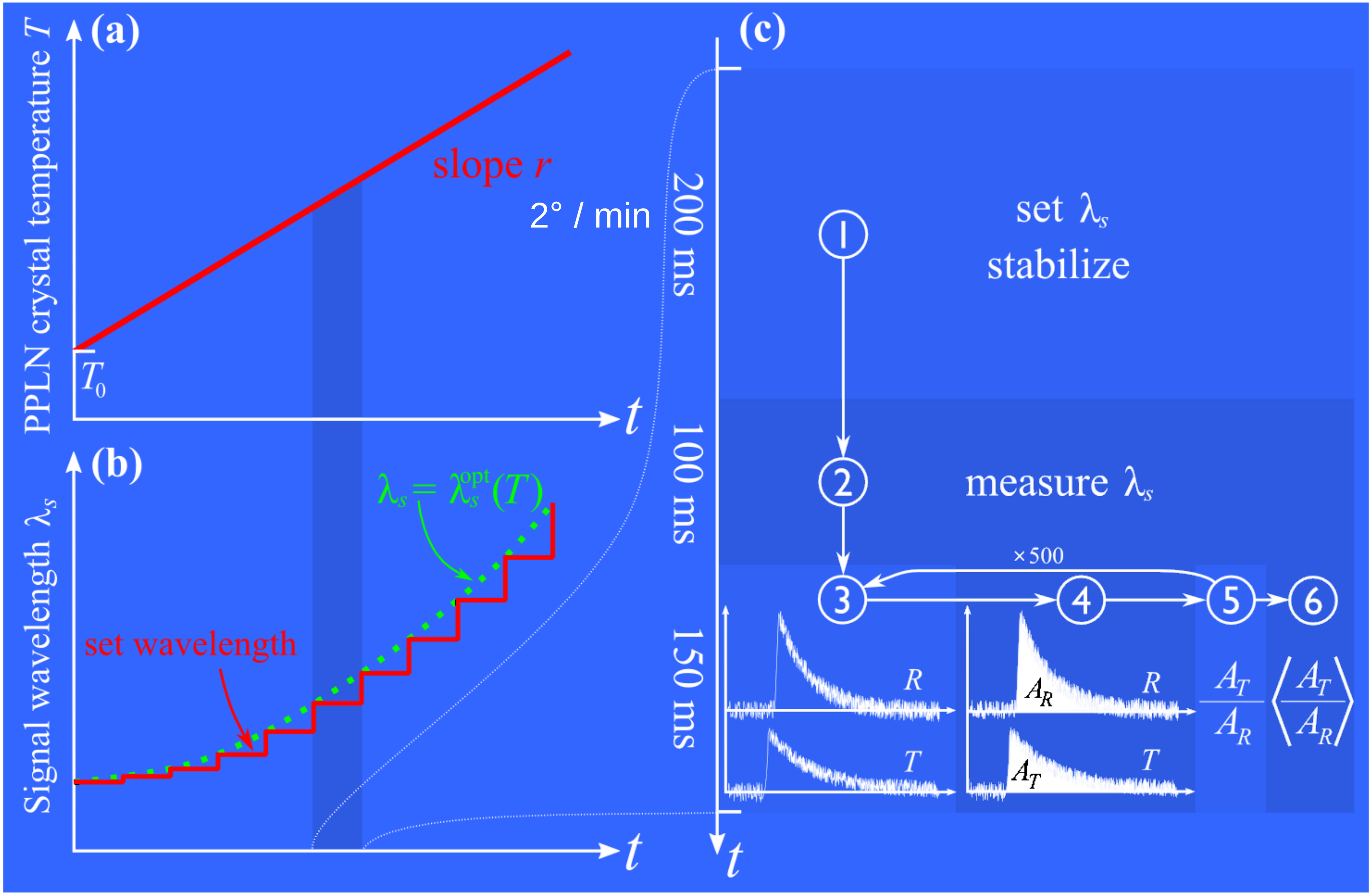
23 Heatable multipass cell
up to 35 m

21/25 Detectors (VIGO)

Idler: 150 μW av.
2817 - 2920 cm^{-1} (29.5 μm)
2900 - 3144 cm^{-1} (29.9 μm)
Step size 0.002 cm^{-1}

Minimum detectable absorption coefficient: 5×10^{-7} cm^{-1} $Hz^{-1/2}$
(few ppm for many compounds of interest)

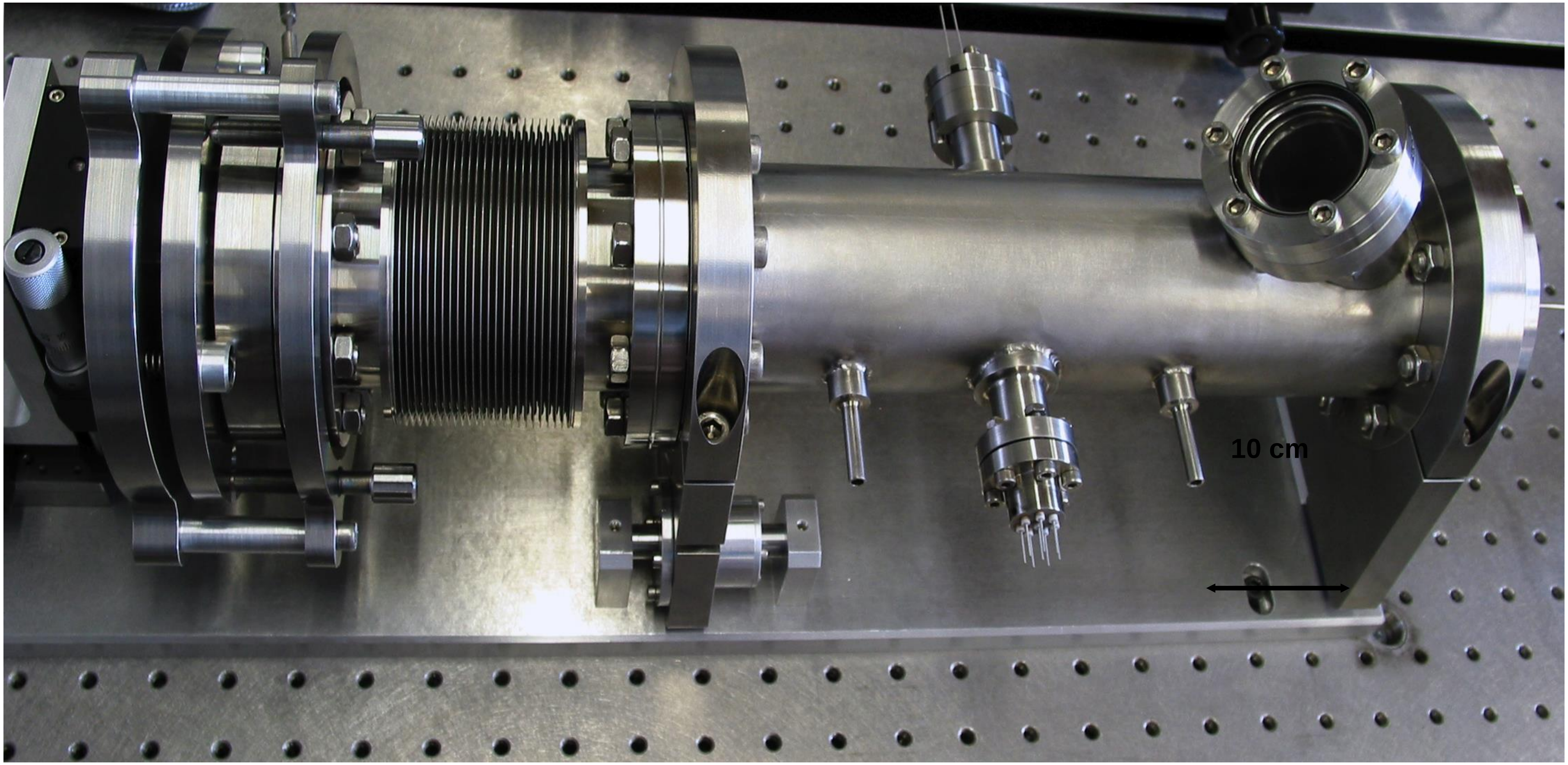
“Timetable” – Tuning of DFG spectrometer



M. Gianella et al.: *Appl. Opt.* **50** (2011)

About 4 cm^{-1} /min with a resolution < 0.02 cm^{-1} (600 MHz)

Fully automatic scan over 250 cm^{-1} in 1 hour, no manual adjustments



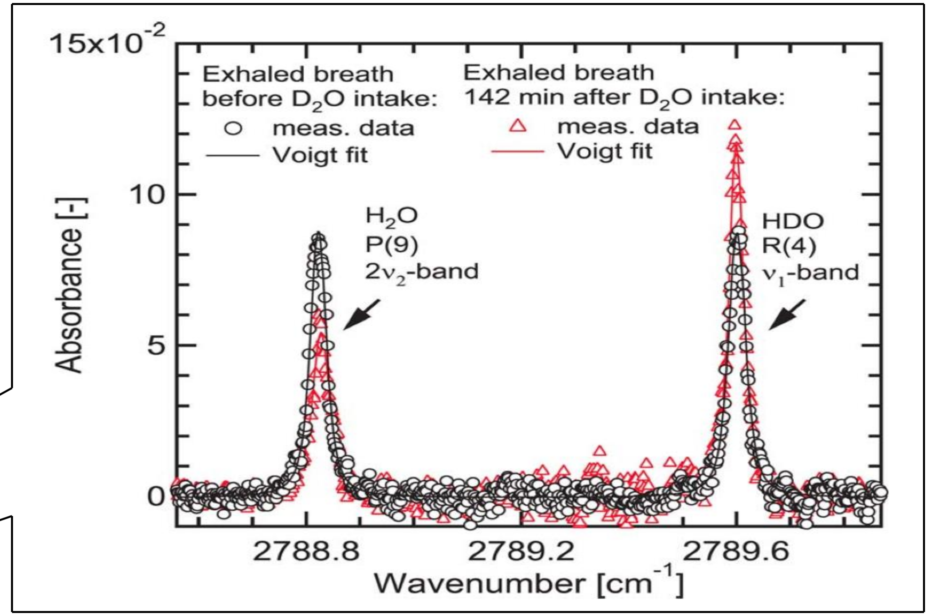
Cell specifications

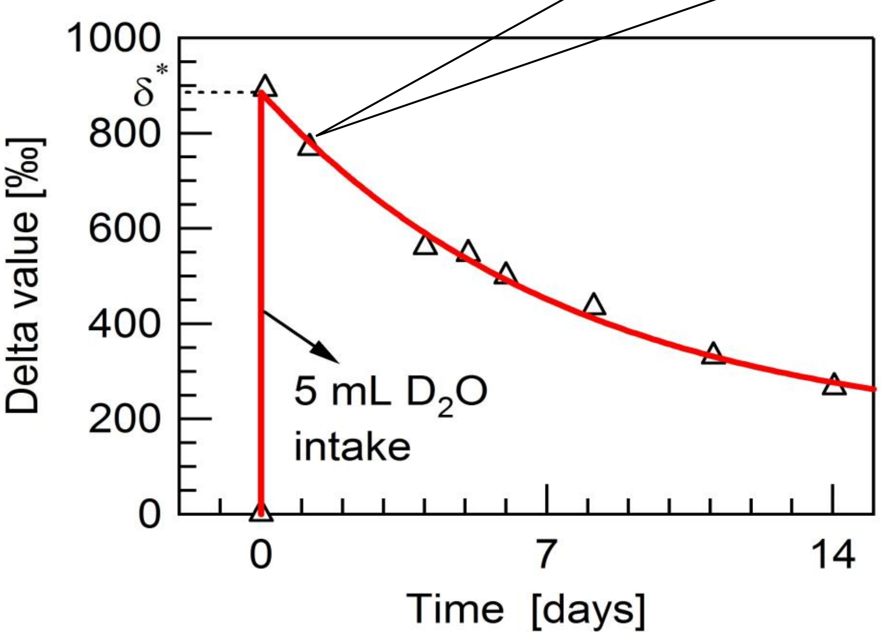
- max temp. 723 K
- 2.0 liters volume, variable pathlength from 9 to 35 m

Time-dependent measurements after heavy water intake

Delta value δD in ‰

$$\delta D = \left(\frac{D/H_{\text{sample}} - D/H_{\text{standard}}}{D/H_{\text{standard}}} \right) \times 1000$$





$$\text{Half-life} = 7.2 \text{ days}$$

Total body water:

$$TBW = \frac{D_2O \text{ mass}}{\delta^* R_{\text{standard}}} = 38.2 \text{ kg}$$

$$= 53.1\ \%\ \text{of}\ 72\ \text{kg}$$

R. Bartlome et al.: *Opt. Lett.* **34**, 866 (2009)

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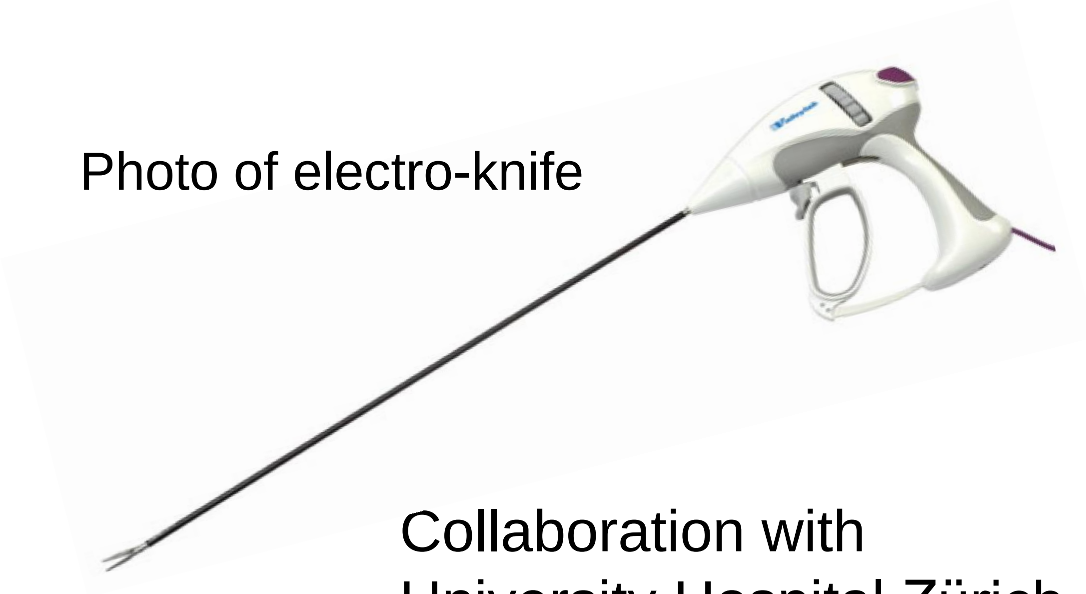
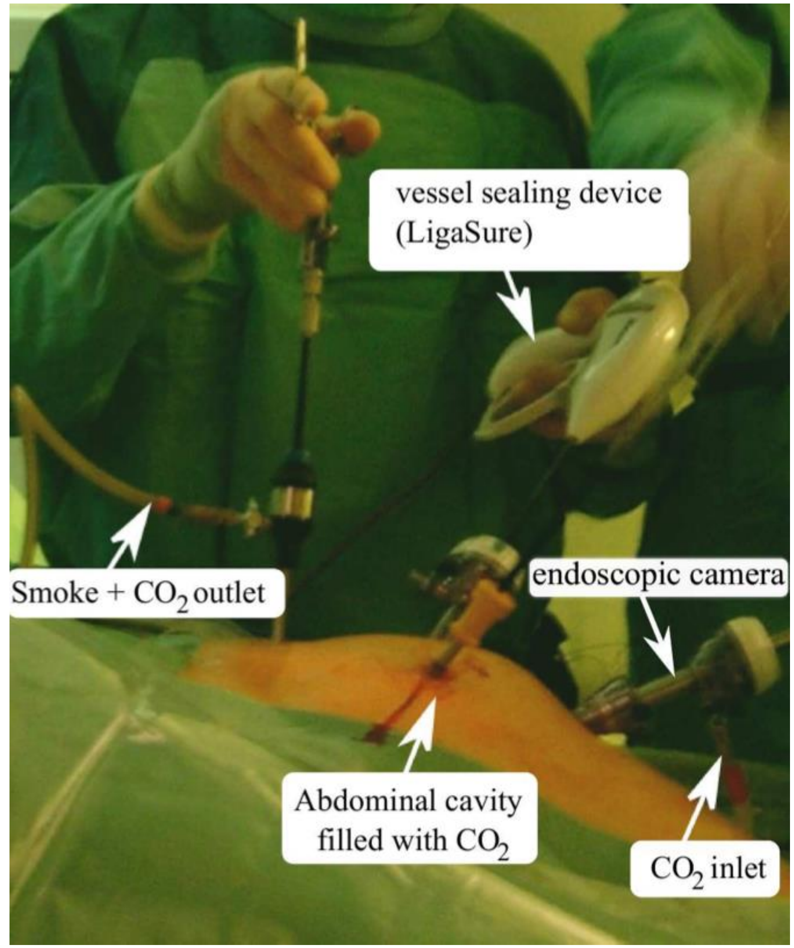
Surgical smoke : *in vivo* studies

Smoke produced during minimal-invasive surgery with electro-knives or lasers. Smoke samples are taken at the hospital, collected in Tedlar bags, followed by laser and FTIR spectroscopic analysis in our lab

Photo of electro-knife

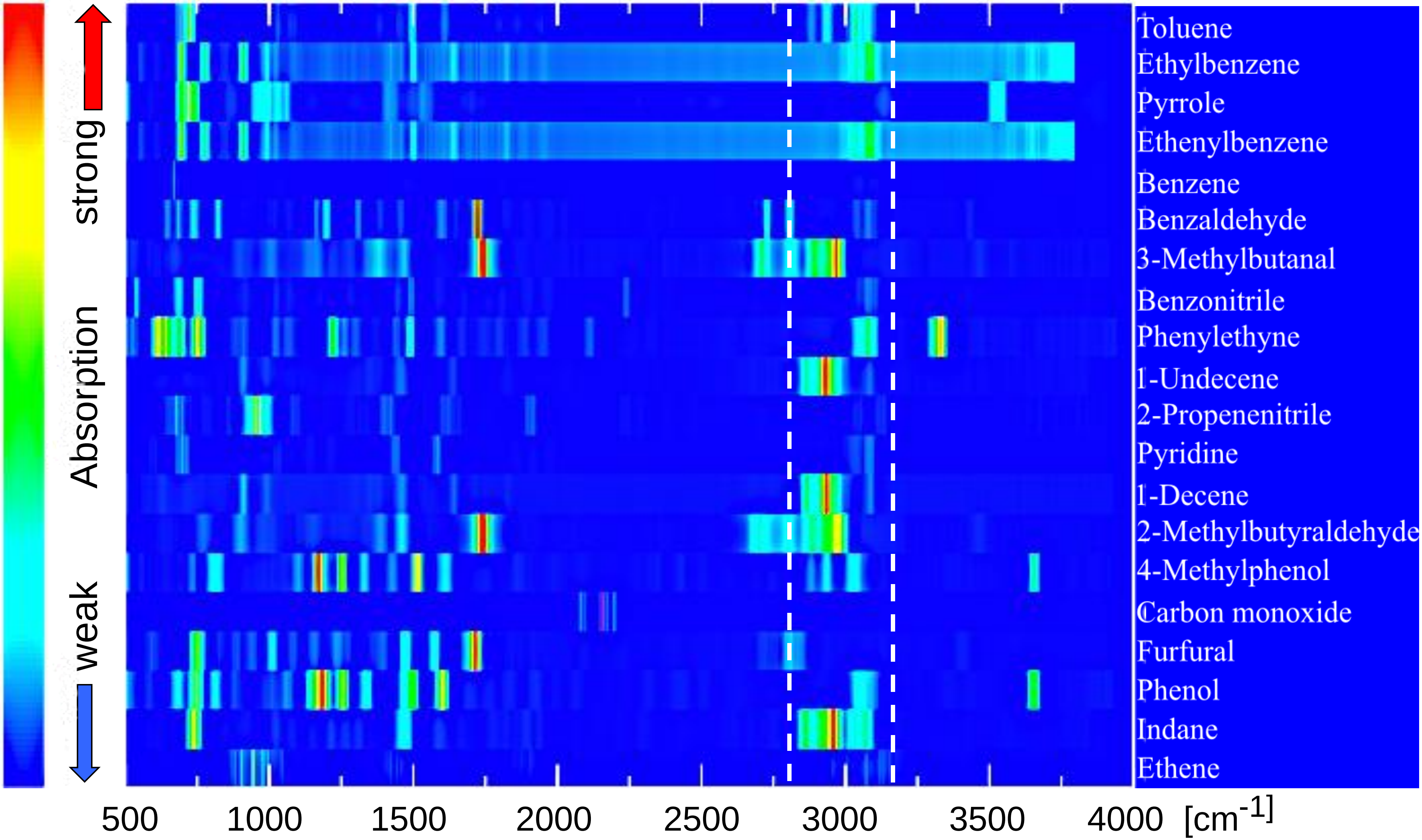


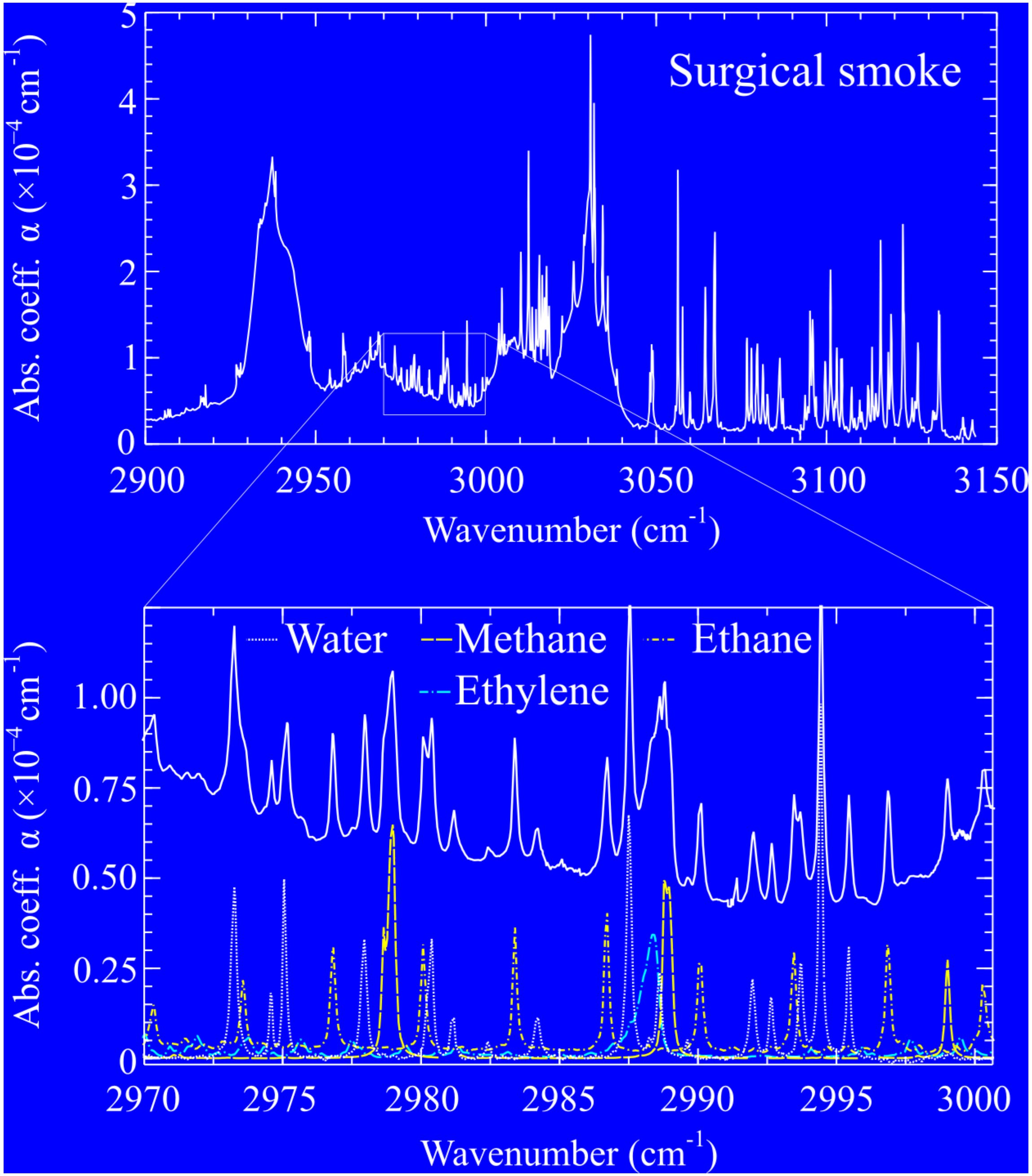
Collaboration with
University Hospital Zürich
(Dr. Dieter Hahnloser)



M. Gianella et al.: *Appl Phys. B* **109**, 485 (2012)
M. Gianella et al.: *Innov. Surgery* (in print)

Absorption ranges of species found in surgical smoke





Spectral analysis of surgical smoke

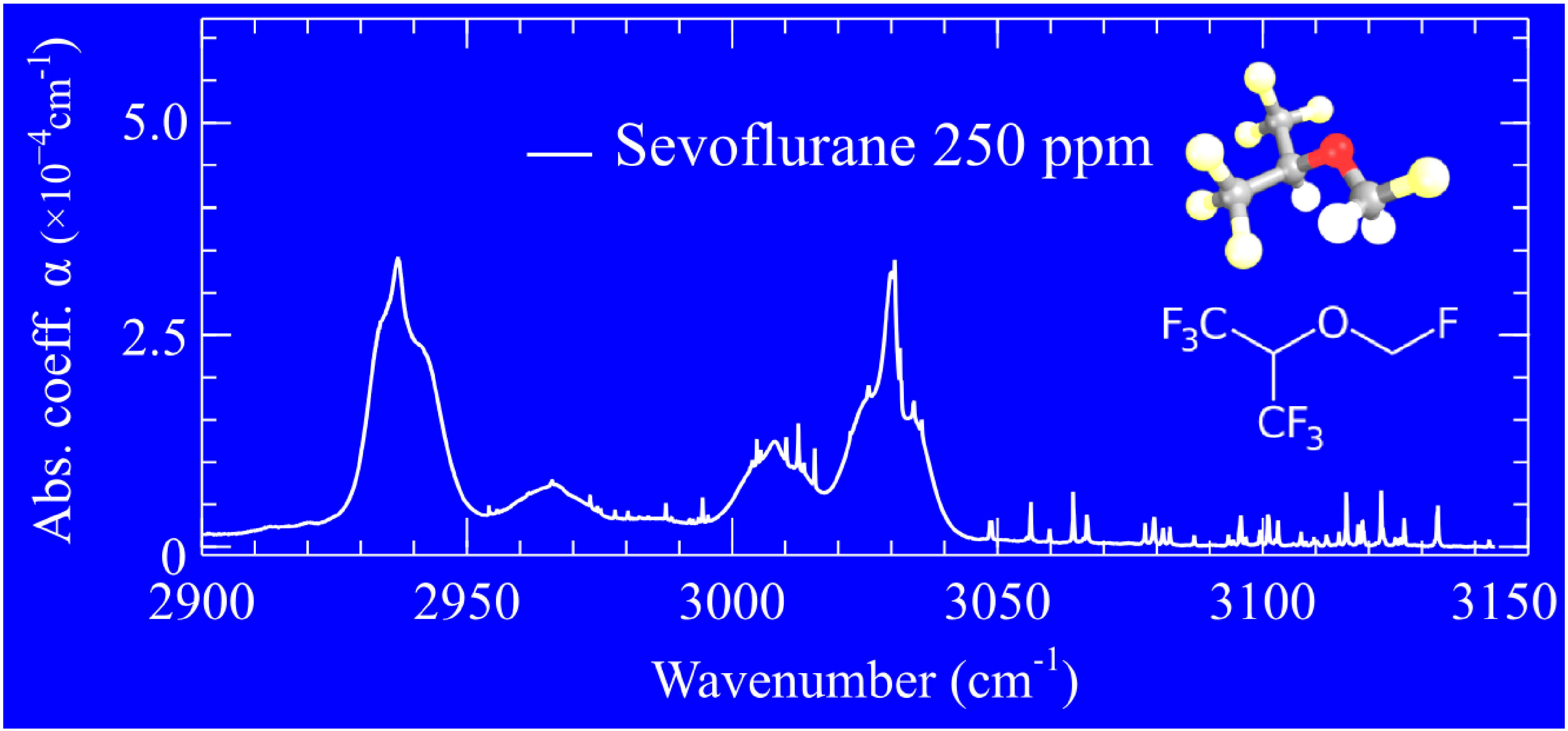
Measured spectrum with DFG spectrometer

Principal Component Analysis (PCA) with improved Mix-Match Algorithm and PNNL database

M. Gianella et al.: *Appl. Spectr.* **63**, 338 (2009)

Spectrum of sevoflurane vapor (anesthetic)

not contained in PNNL database, recorded with **DFG spectrometer**
(250 ppm, p=950 mbar, T = 25 °C)

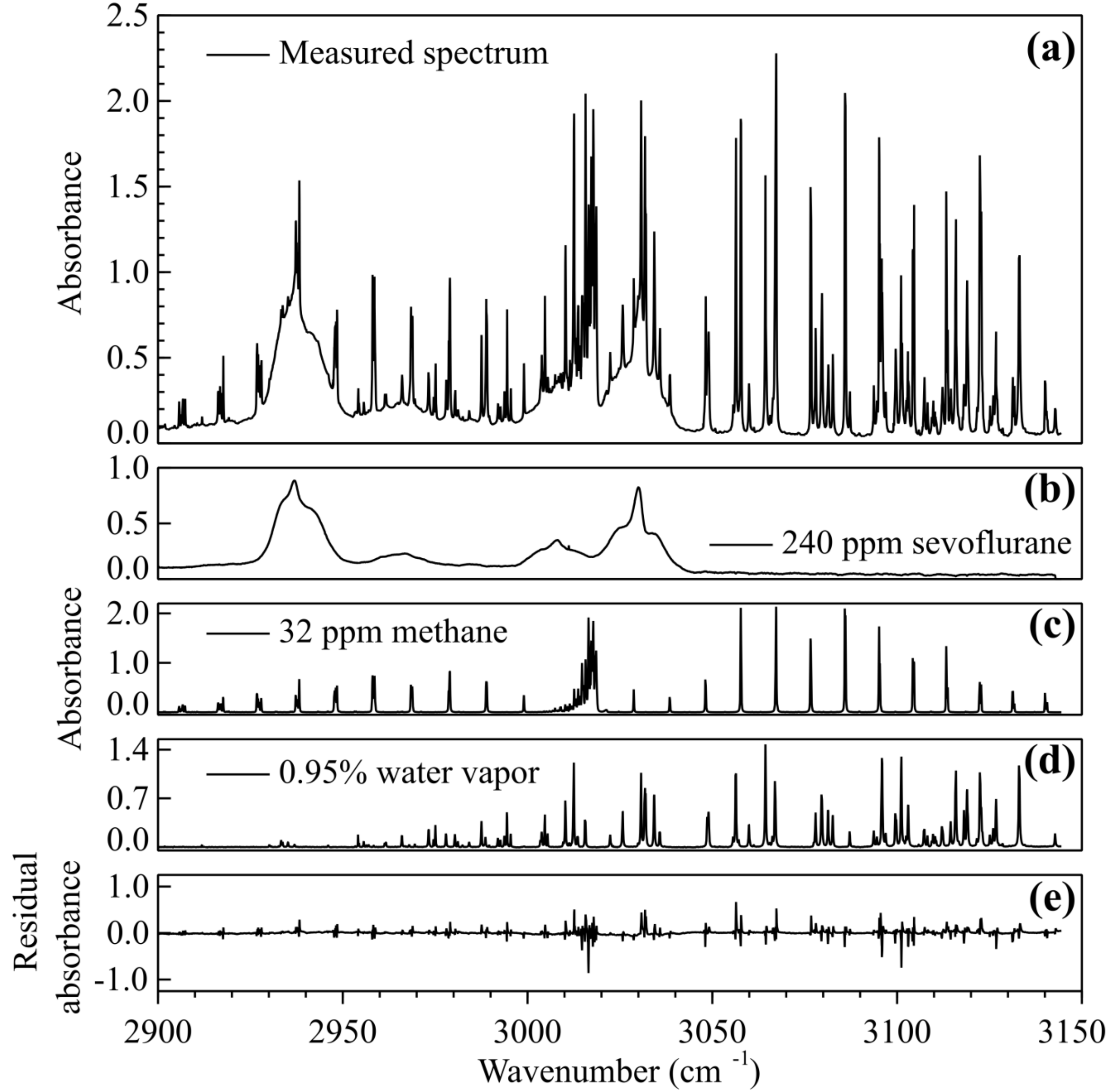


Analysis of surgical smoke

Spectrum of **surgical smoke** collected during **laparoscopic surgery** **(a)**

Spectra of its components **(b) — (d)**

Residual spectrum **(e)**



Detection limits (c_{min}) and recommended exposure limits (REL)

Substance	c_{min} ppm	REL ppm
(Cyclo) alkanes, -enes, -ynes		
methane	1.0	10000
ethane	1.0	10000
propane	1.6	1000
butane	2.2	800
pentane	2.1	600
hexane	2.0	50
octane	1.4	300
nonane	1.1	200
decane	0.9	—
undecane	0.8	—
ethylene	5.0	10000
1,3-butadiene	18	5
hexene	3.8	—
cyclohexane	0.5	200
cyclohexene	1.4	300
Amines		
methylamine	10	10
dimethylamine	6.4	2
trimethylamine	3.8	2

Substance	c_{min} ppm	REL ppm
Nitriles		
acetonitrile	32	20
acrylonitrile	150	2
(Cyclo) alcohols		
methanol	7.8	200
ethanol	6.9	500
propanol	4.5	200
isopropanol	3.0	200
cyclohexanol	1.2	50
Aldehydes		
formaldehyde	0.78	0.3
acetylaldehyde	50	50
benzaldehyde	15	—
acrolein	65	0.1
Carboxylic acids		
formic acid	7.7	5
acetic acid	32	10

Substance	c_{min} ppm	REL ppm
Inorganics		
hydrogen chloride	0.92	
nitrogen dioxide	11	3
water	450	—
hydrogen fluoride	0.0001	1
Aromatic compounds		
benzene	8.7	0.5
m-xylene	7.7	100
o-xylene	7.6	100
p-xylene	6.5	100
styrene	11	20
toluene	8.8	50
furan	130	—
pyridine	12	5
Others		
acetone	14	500
dichloromethane	67	50
nicotine	2.7	0.07
sevoflurane	20	2
ammonia	750	20
carbon monoxide		30

→ Many **compounds** can be detected below REL if present in surgical smoke

Chemical composition of *in vivo* samples

Gaseous component	Average concentrations	REL Recommended Exposure limit	Detection limit
Water vapor (H_2O)	0.7%	----	120 ppm
Methane (CH_4)	2.3 ppm	10’000 ppm	0.3 ppm
Ethane (C_2H_6)	≈ 1 ppm (3 of 16 samples)	10’000 ppm	0.3 ppm
Ethylene (C_2H_4)	≈ 8 ppm (2 of 16 samples)	10’000 ppm	2 ppm
Carbon monoxide (CO)	2 ppm	30 ppm	0.25 ppm (Diode laser 2.3 μm)
Sevoflurane ($C_4H_3F_7O$) (anesthetic gas)	60 – 450 ppm	2 ppm	5 ppm

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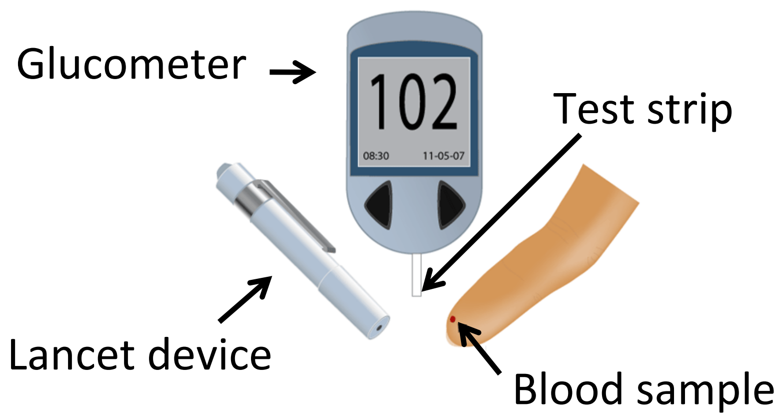
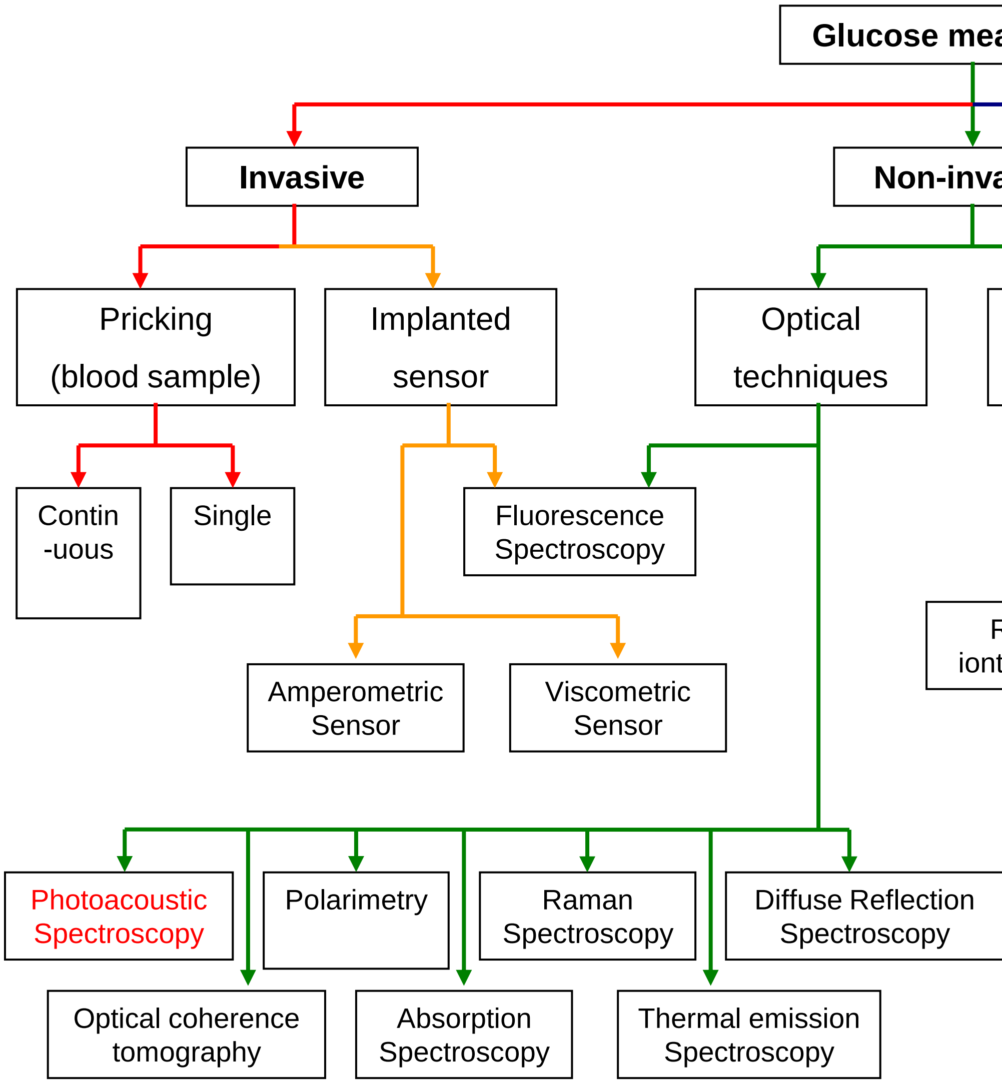
Diabetes as a human metabolic disease

- Patients need to measure the blood sugar level several times a day
- Glucose level of a healthy human: 65 – 120 mg/dl
- Common blood sugar measurements are invasive



- **Goal**: Development of a non-invasive glucose sensor based on mid-IR laser, photoacoustic detection and glucose monitoring in interstitial fluid through skin

Glucose sensing techniques



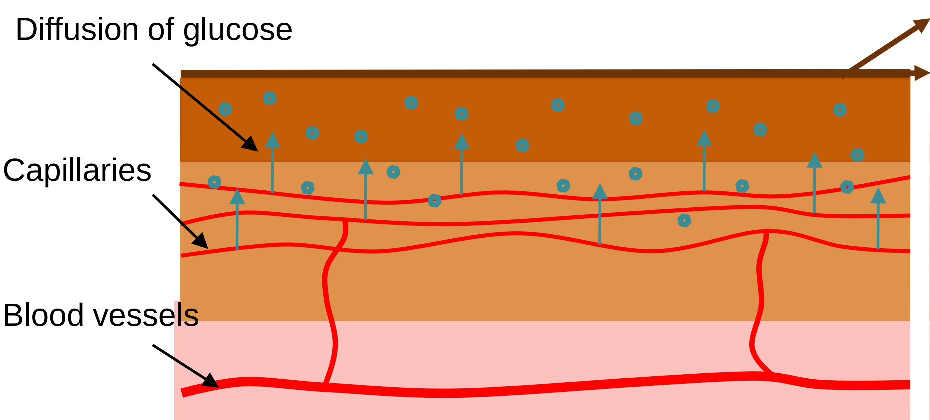
J. Kottmann *et al.*, *Biomed. Opt. Exp.* **3**, 667 (2012)
C. E. F. do Amaral *et al.*, *Med. Eng. Phys*. **30**, 541 (2008)

Interstitial fluid glucose sensing

MIR light: Strong glucose absorption, less interference than in NIR
=> Opt. penetration < 100 µm
=> blood vessels are not reached

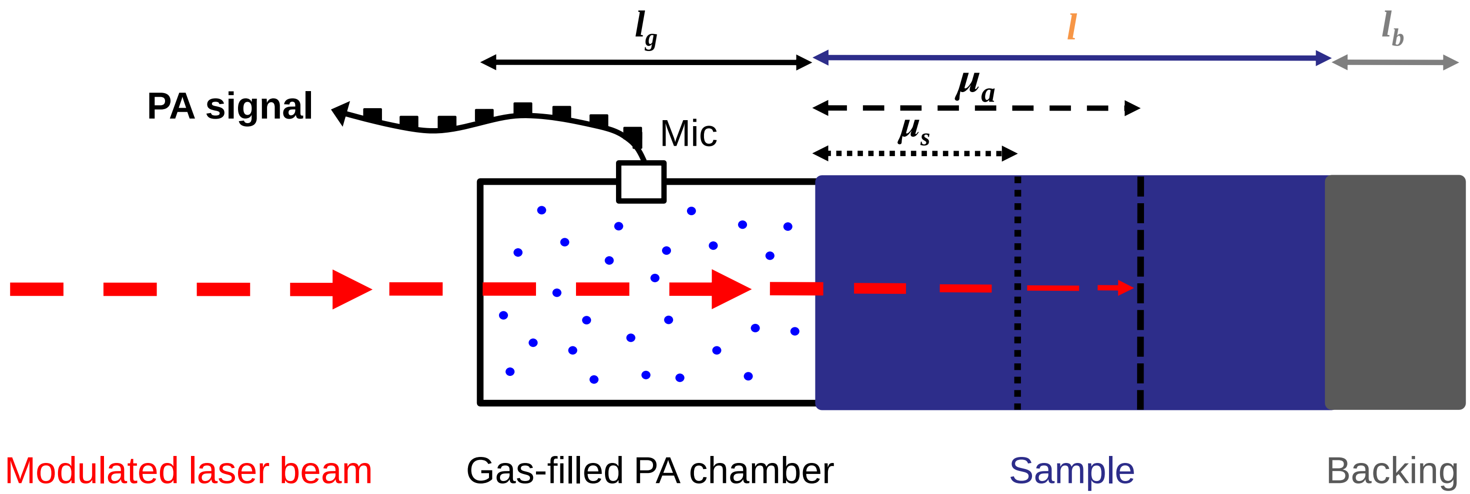
Epidermis: **=>** Glucose diffusion into epidermal interstitial fluid
=> Correlation with blood glucose
=> No glucose in the stratum corneum

QCL and
PA technique } sensitive technique with potential for miniaturisation



Thickness [mm]	Skin layer	Water [%]	Glucose
0.01 – 0.02	**Stratum corneum**	≈ 10	no
0.1 – 0.3	**Epidermis**	≈ 60	Interstitial fluid
1 – 2	**Dermis**	≈ 60	Blood /interstitial fluid
2 – 4	**Subcutaneous tissue**	≈ 60	Interstitial fluid

Photoacoustic detection: Indirect PA signal generation



I Intensity modulated with frequency *f*

$$\mu_s = \left(\frac{D_s}{\pi f}\right)^{\frac{1}{2}} \text{ with } D_s = \text{ thermal diffusivity}$$

$$\mu_a = \frac{1}{\alpha} \text{ with } \alpha = \text{ absorption coefficient}$$

F depends on cell and coupling gas parameters

Gas piston model

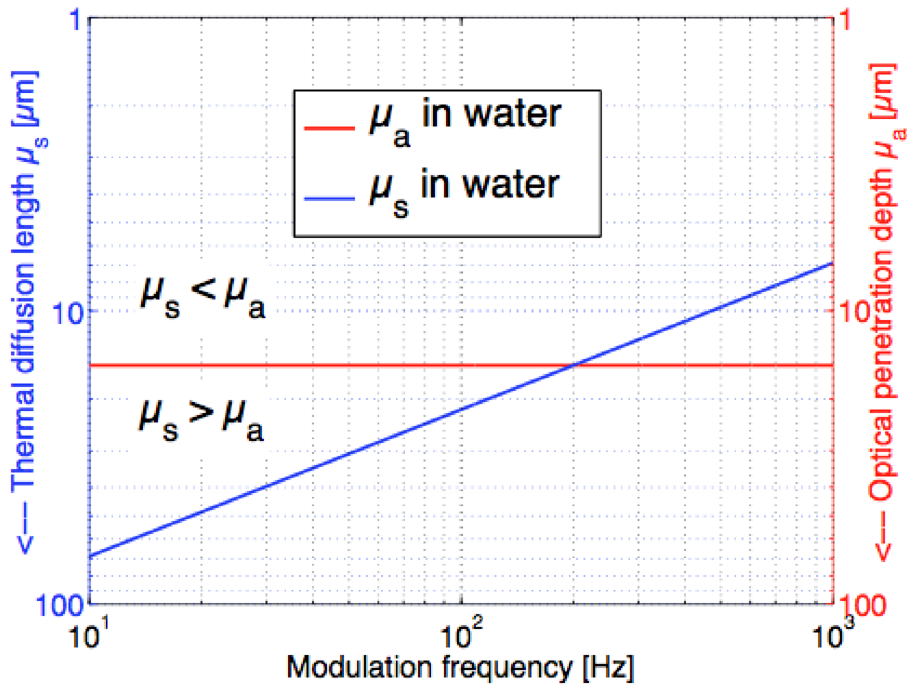
Case	Length ratio	Characteristics	Approx. PA signal amplitude
1.	$\mu_s > \mu_a \gg l$	Opt. and therm. thin	$(1-i)\alpha l(\mu_b/k_b)F$
2.	$\mu_a > \mu_s > l$	Opt. and therm. thin	$(1-i)\alpha l(\mu_b/k_b)F$
3.	$\mu_a \gg l > \mu_s$	Opt. thin, therm. thick	$-i\alpha\mu_s(\mu_s/k_s)F$
4.	$\mu_s \gg l \gg \mu_a$	Opt. thick, therm. thin	$(1-i)(\mu_b/k_b)F$
5.	$l \gg \mu_s > \mu_a$	Opt. and therm. thick	$(1-i)(\mu_s/k_s)F$
6.	$l \gg \mu_a > \mu_s$	Opt. and therm. thick	$-i\alpha\mu_s(\mu_s/k_s)F$

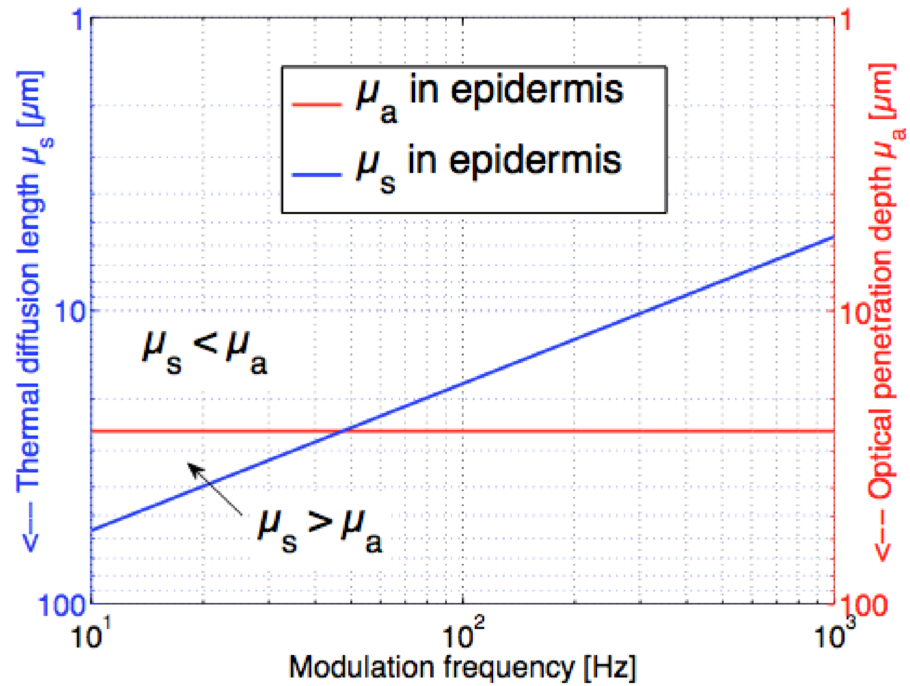
A. C. Tam, *Rev. Mod. Phys*. **58**, 381 (1986)

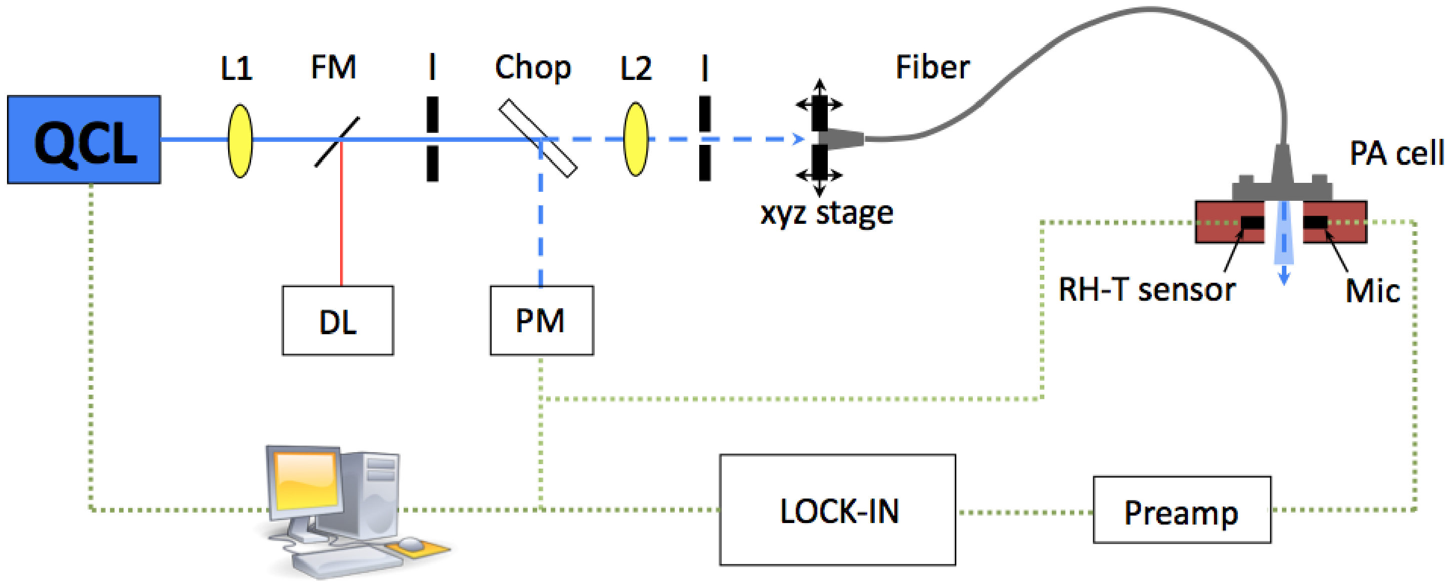
Photoacoustic detection in skin

Case 5 ($\mu_s > \mu_a$): $S \propto \frac{I_0}{V \cdot f}$

Case 6 ($\mu_s < \mu_a$): $\boxed{S \propto \frac{I_0 \cdot \alpha}{V \cdot f^{3/2}}}$ $\Rightarrow$ f > 50 Hz for epidermis, f > 200 Hz for water

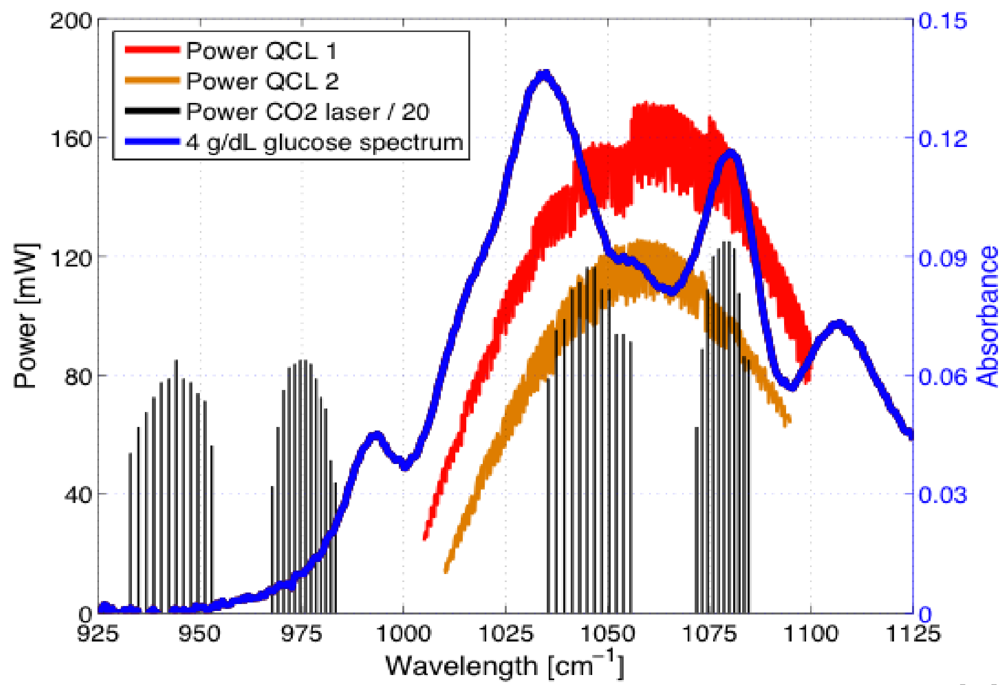






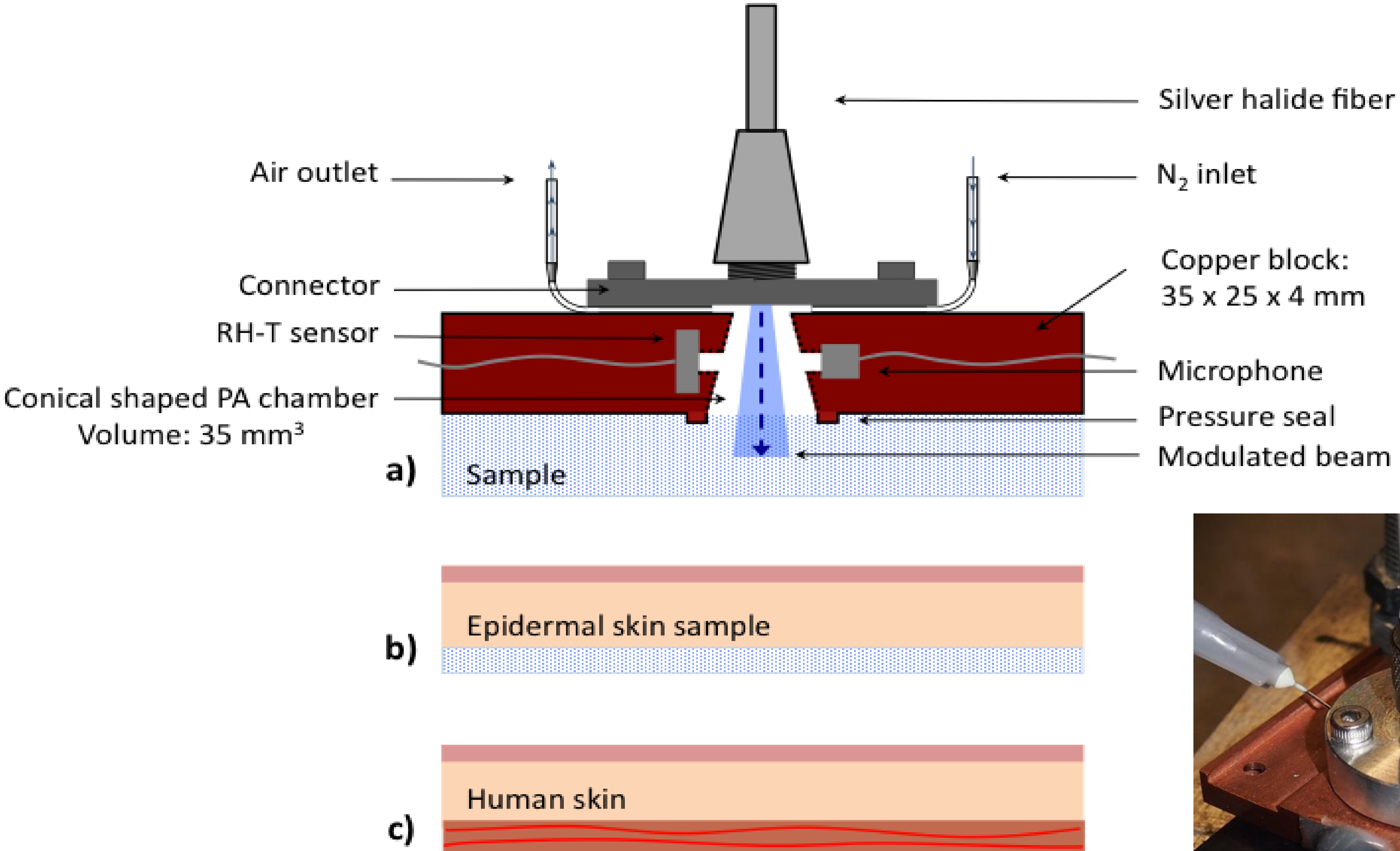
Setup with EC-QCL and fiber-coupled PA cell

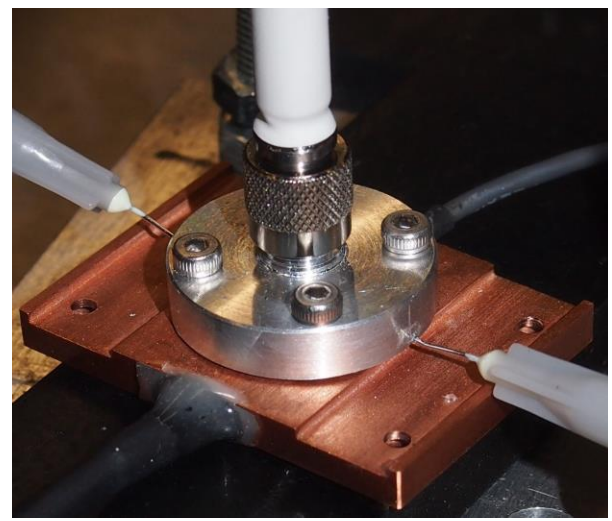
Glucose spectrum and QCL tuning range



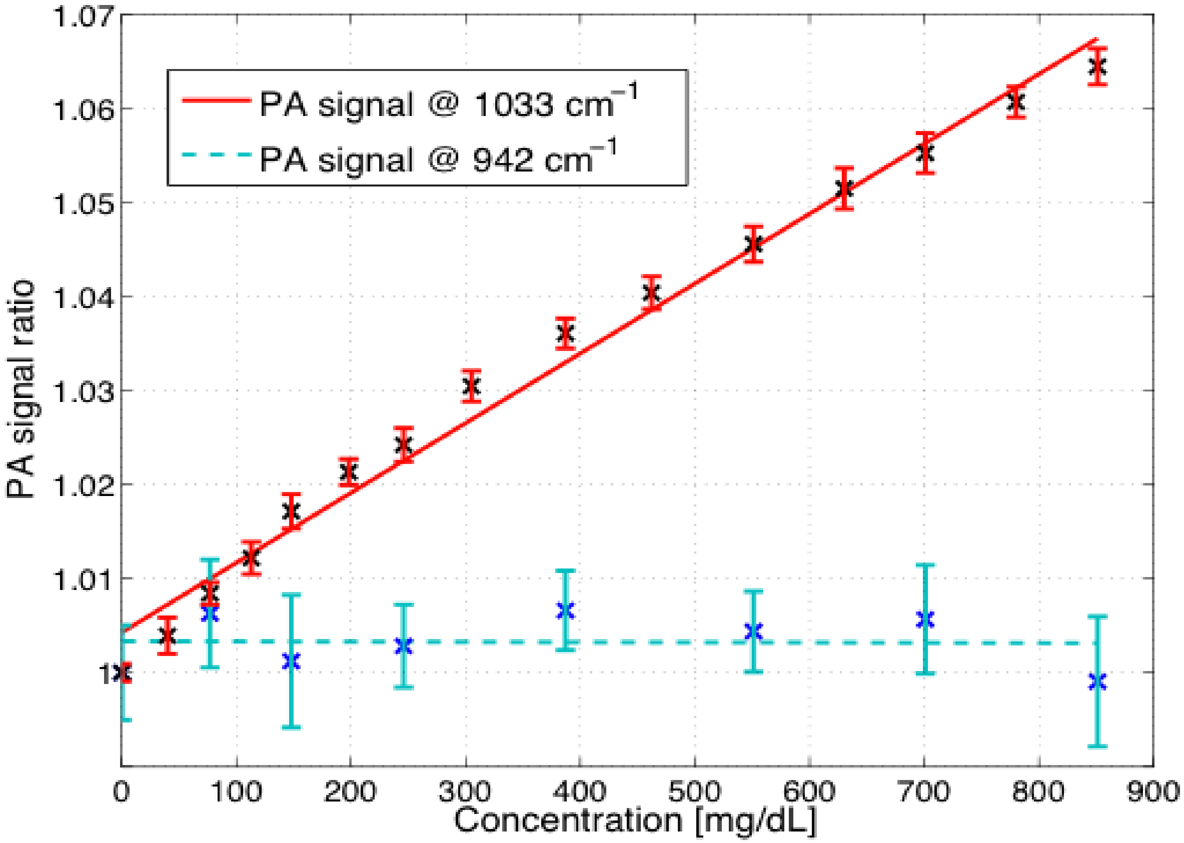
Fiber-coupled PA cell

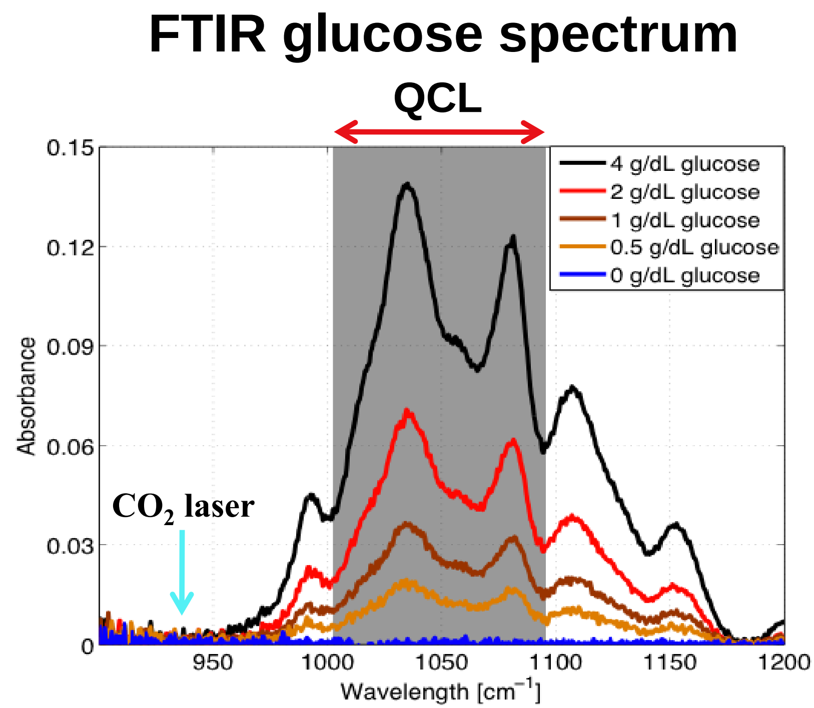
with N_2 ventilation and relative humidity monitoring





Glucose detection in aqueous solutions and gels

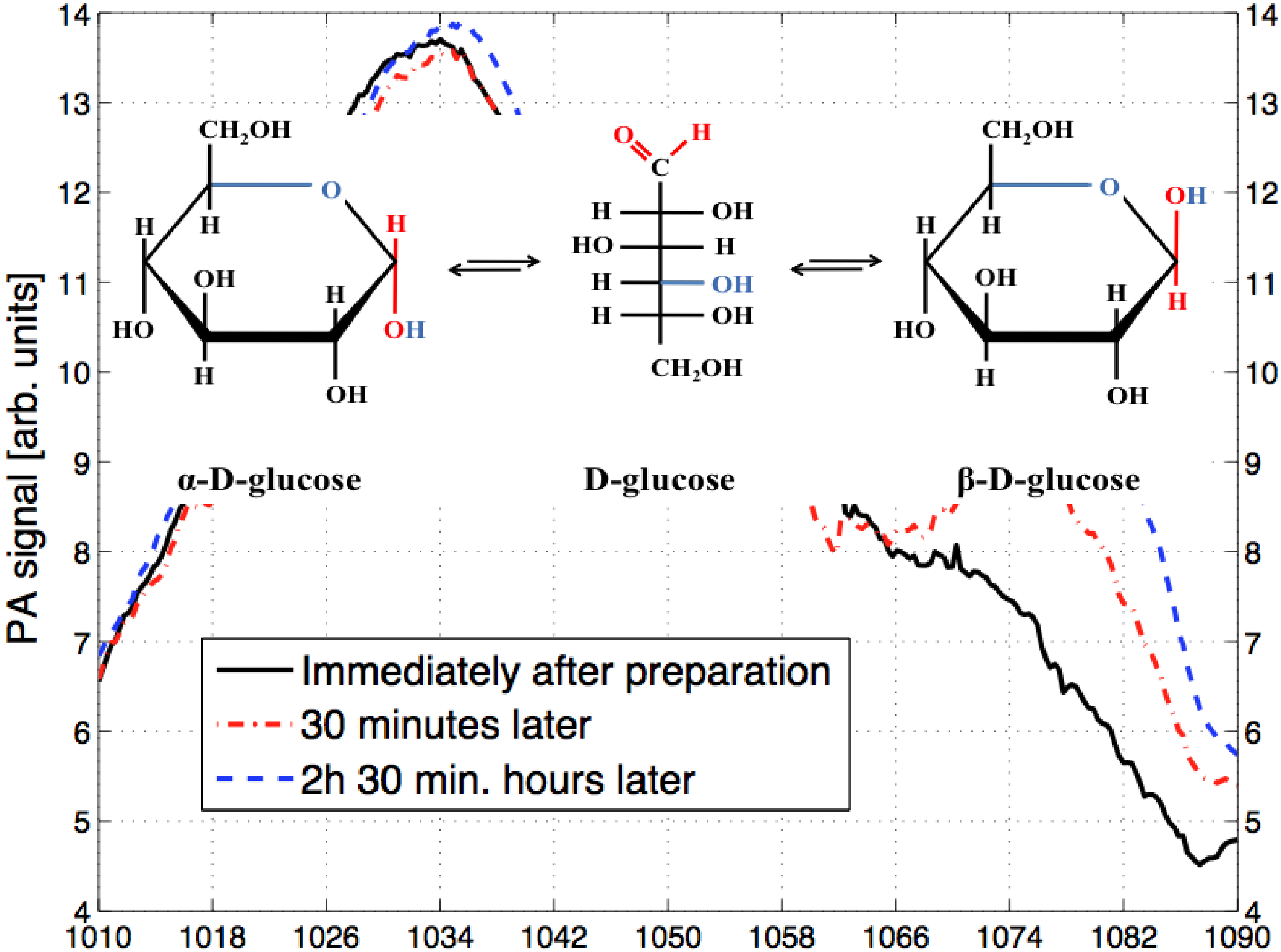




- Detection limit: 30 mg/dL for a SNR=1
- From FTIR spectrum: Glucose absorption peaks at 1034 and 1080 cm^{-1}

J. Kottmann *et al*., *Rev. Sci. Instrum.*, **82**, 084903 (2011)

Aqueous glucose solution: Mutorotation

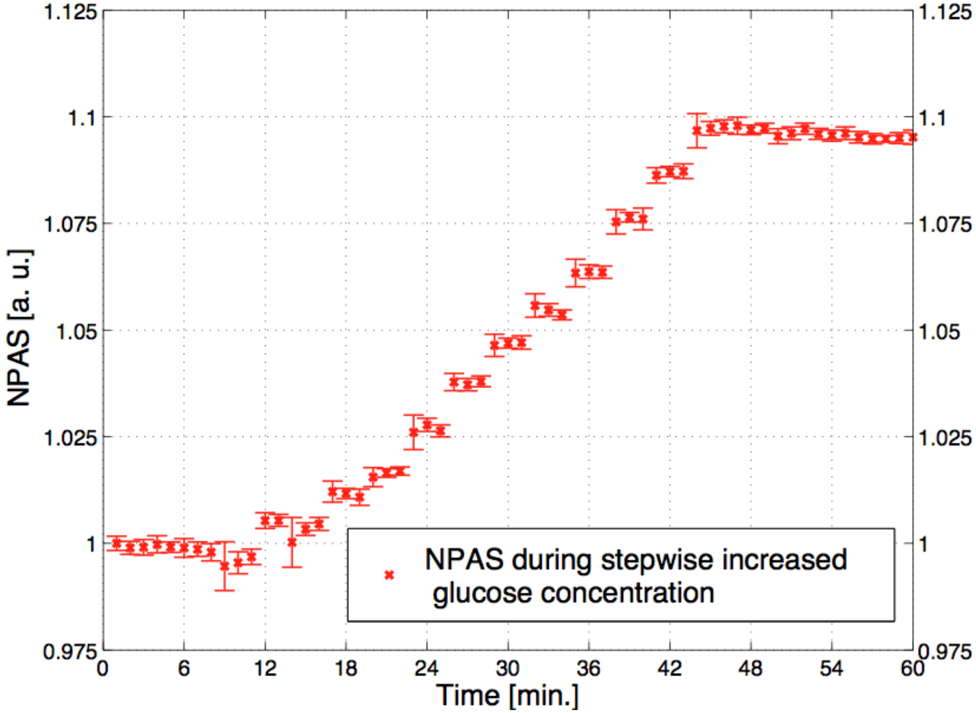


Time dependent spectral changes of α-D-glucose powder dissolved in water due to Mutorotation

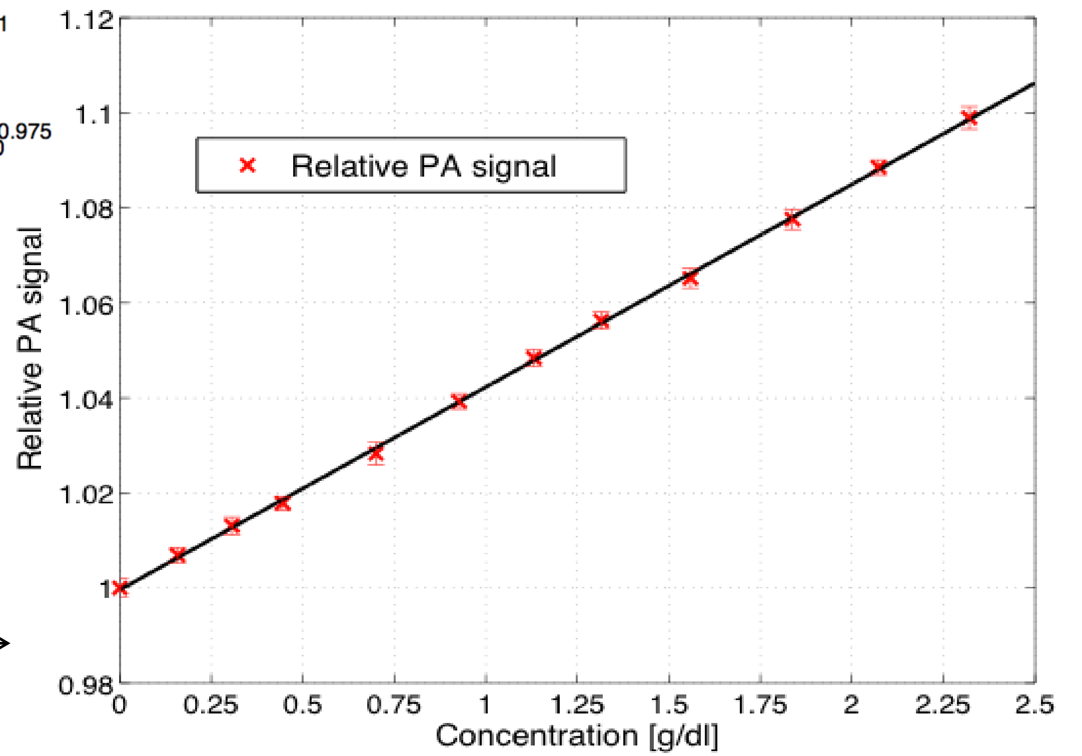
Equilibrium in water

EC-QCL tuned in 0.9 cm^{-1} steps with external grating

Glucose in keratinocyte solution
(measured with flow cell and diamond)

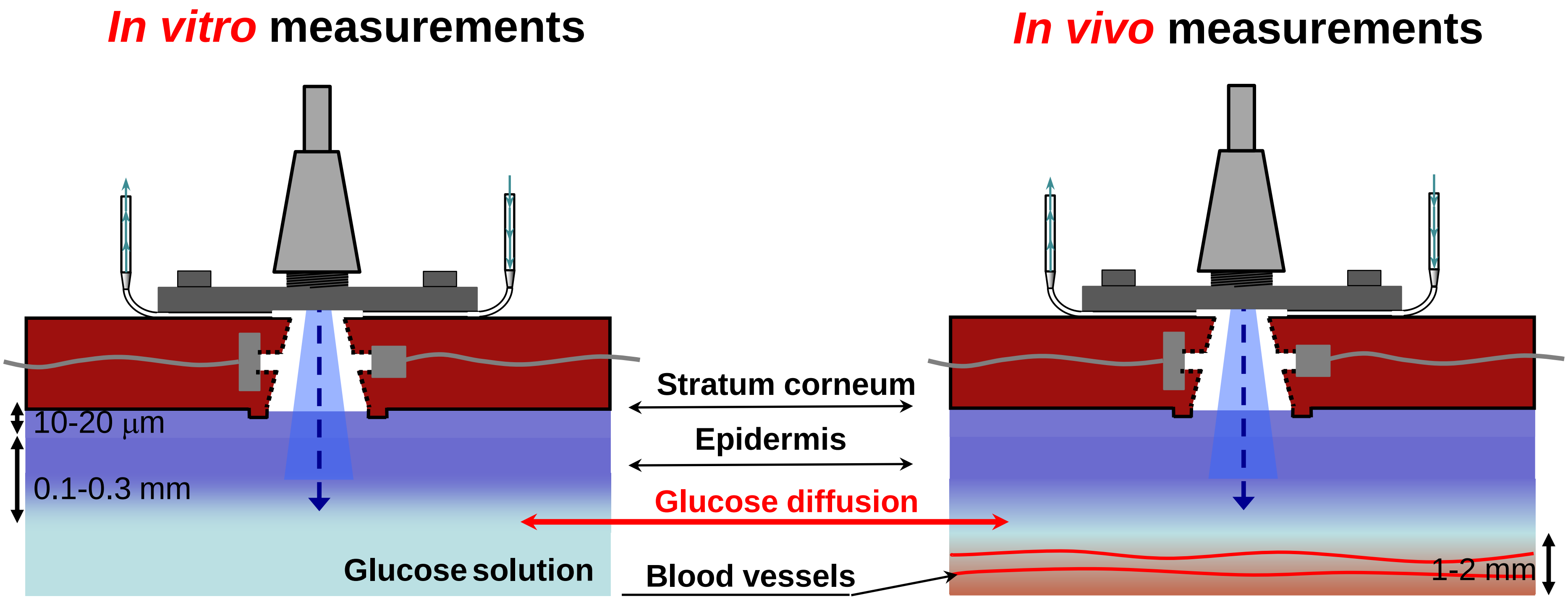


Normalized PA signal of keratinocyte solution with stepwise increase of glucose concentration



Normalized PA signal at 1034 cm^{-1} vs glucose concentration in aqueous solution (cf. physiological range: 30-500 mg/dl)

Glucose detection in epidermal skin

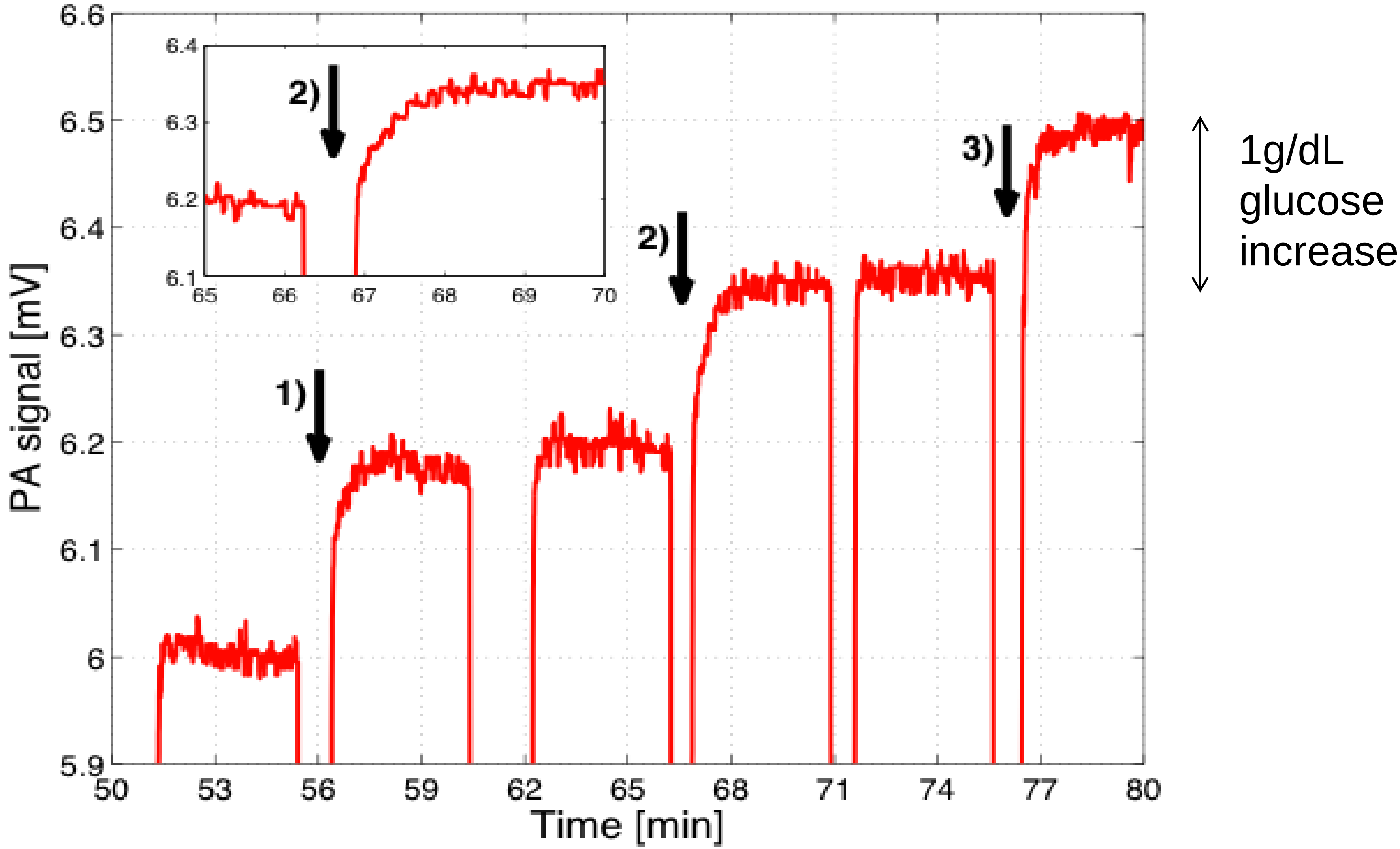


The epidermal glucose concentration is altered through passive diffusion

Diffusion out of the glucose solution

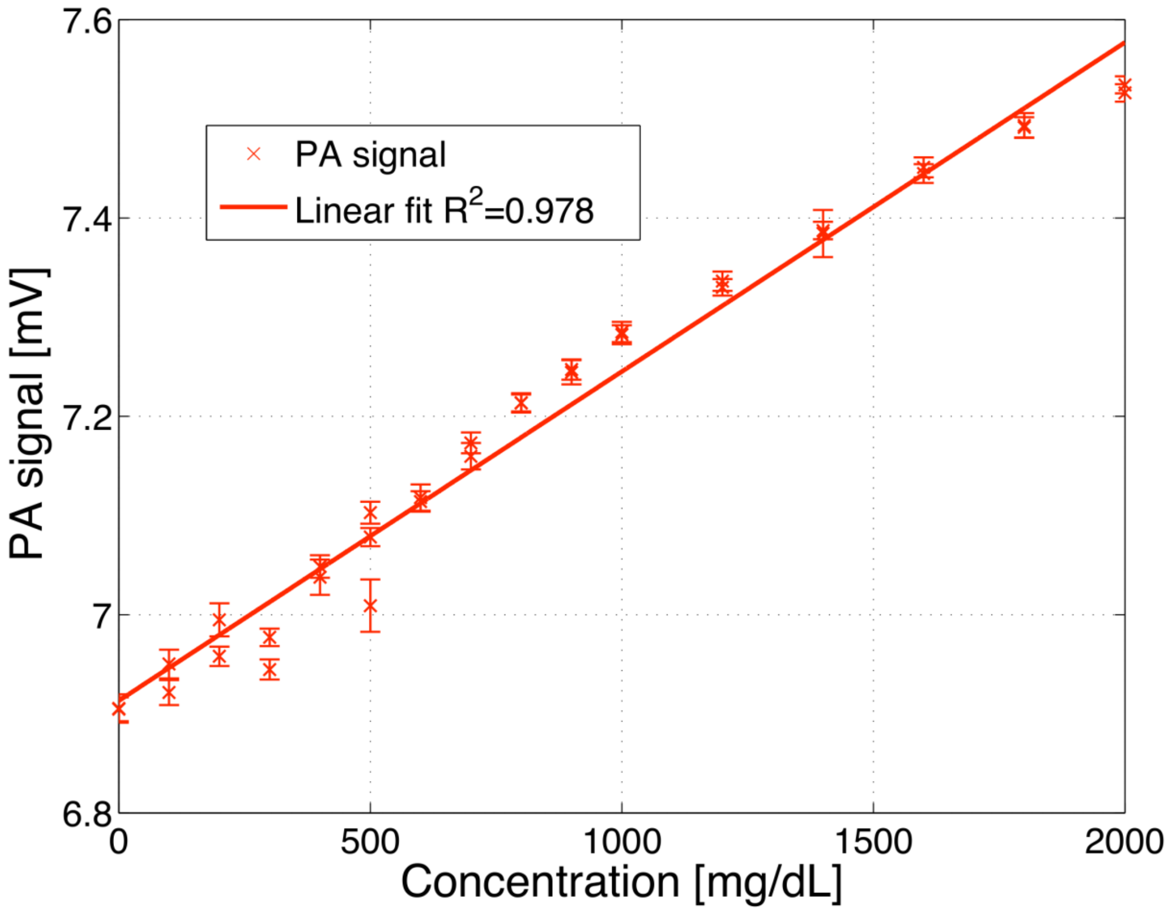
Diffusion out of the blood vessels

Diffusion process of glucose into epidermal skin



J. Kottmann et al.: *Biomed. Opt. Express* **3**, 667 (2012)

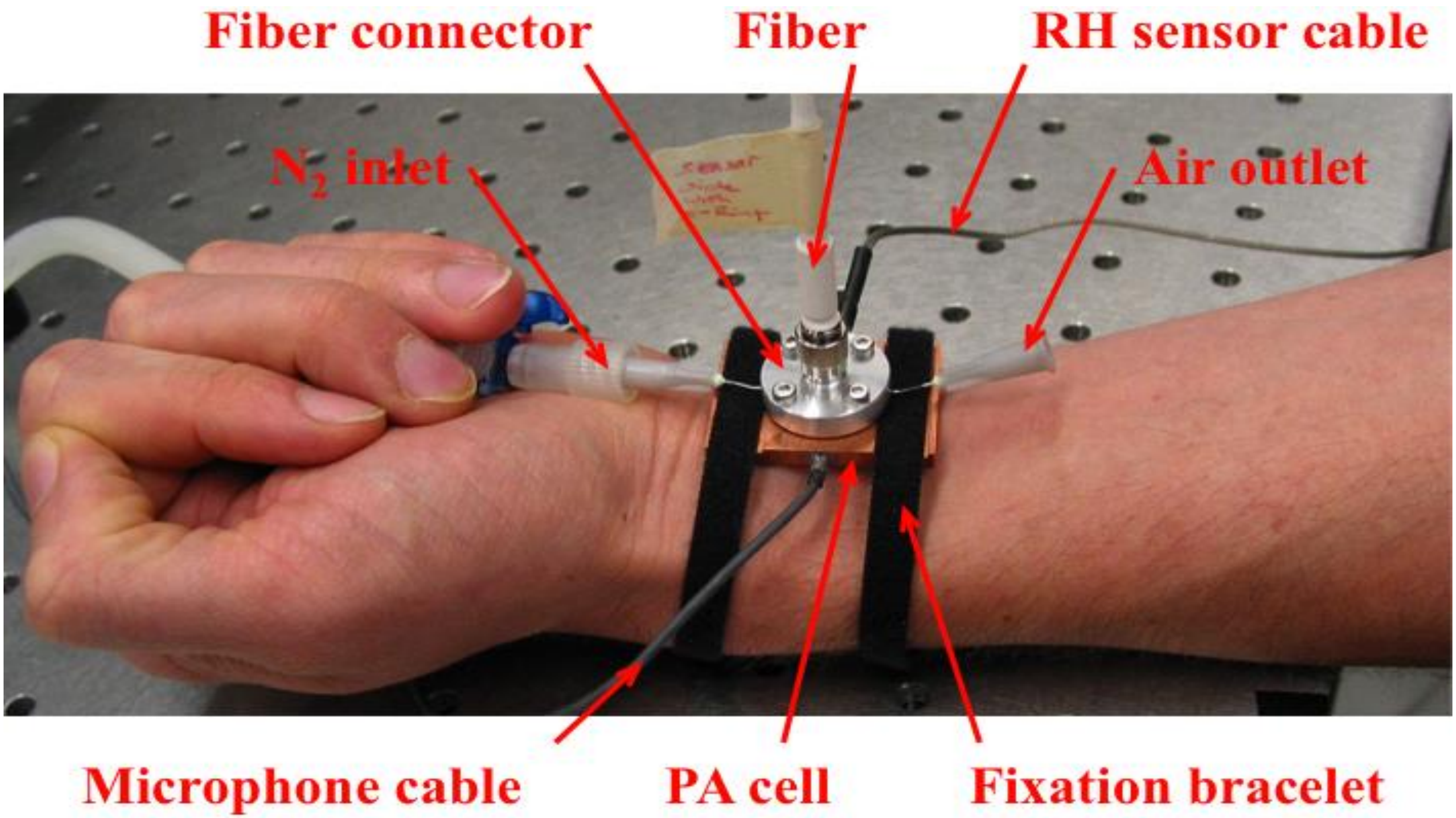
In vitro glucose detection in epidermal skin sample



Measured at 1034 cm^{-1} with 32 mW average power

- Detection limit **≤ 100 mg/dL** for SNR = 1 (physiological range of 65-120 mg/dL)
- Linear dependence from 0 – 10 g/dl

In vivo measurements at the forearm

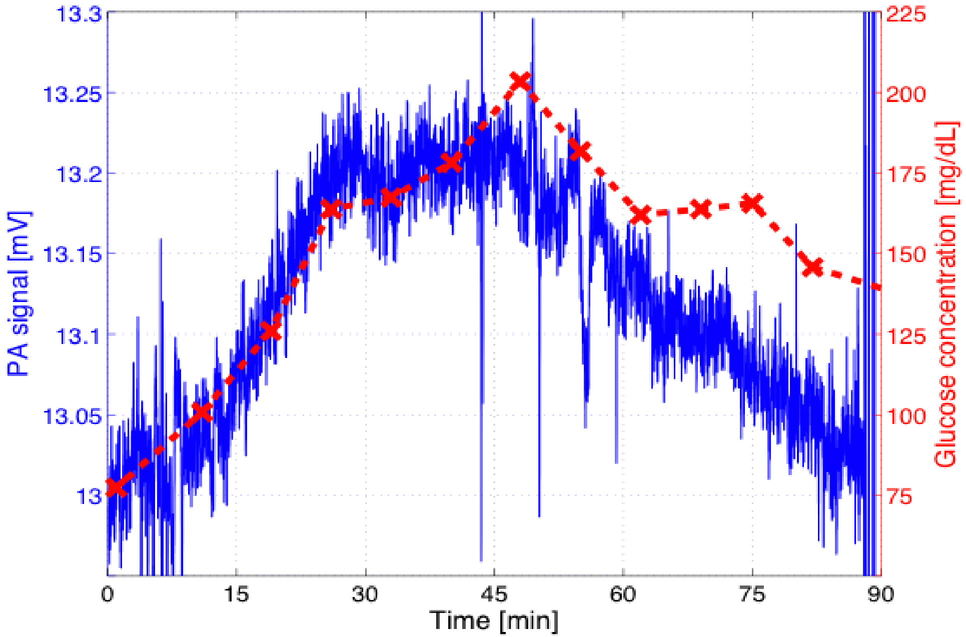


J. Kottmann et al.: *Sensors* **13**, 535 (2013)

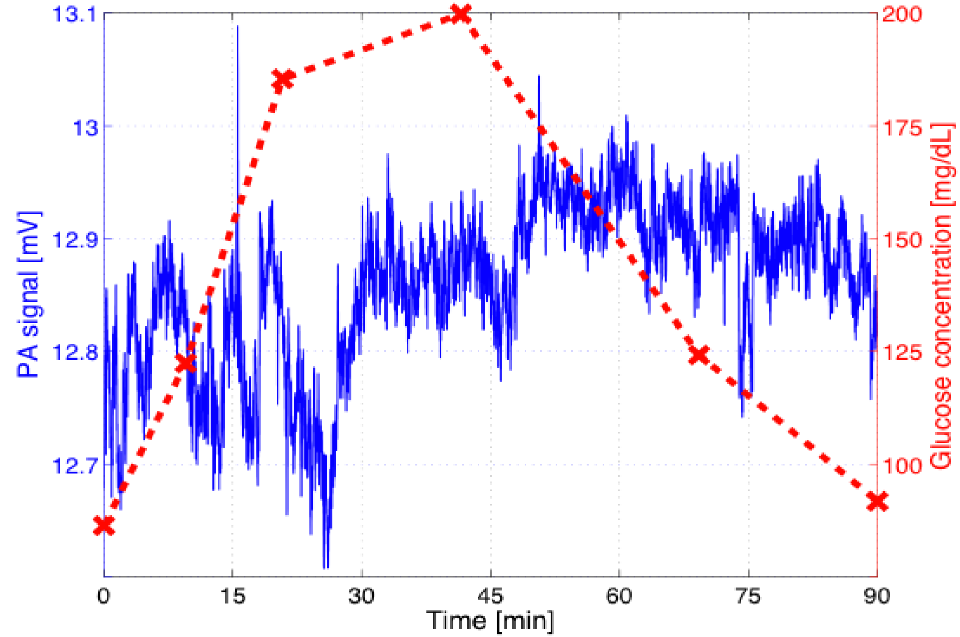
In vivo glucose measurements

Oral glucose tolerance test

Measurement 1



Measurement 2



PA signal Invasive

PA signal Invasive

Corrected for RH & T variations, sensitive to arm movements

Qualitative correlation between invasive and non-invasive glucose measurement

Not always clear correlation between the invasive and non-invasive measurement

Outline

- Introduction: mid-IR laser spectroscopic sensing
- Breath analysis: D/H isotope ratio measurement
- Surgical smoke analysis
- Non-invasive glucose sensing: QCL and PAS
- Cocaine detection in saliva: FTIR and QCL
- Conclusions and outlook

Detecting cocaine in drugged drivers today

Stopping car due to initial suspicion

collect Saliva

checking for drugs

Negative: driver continues

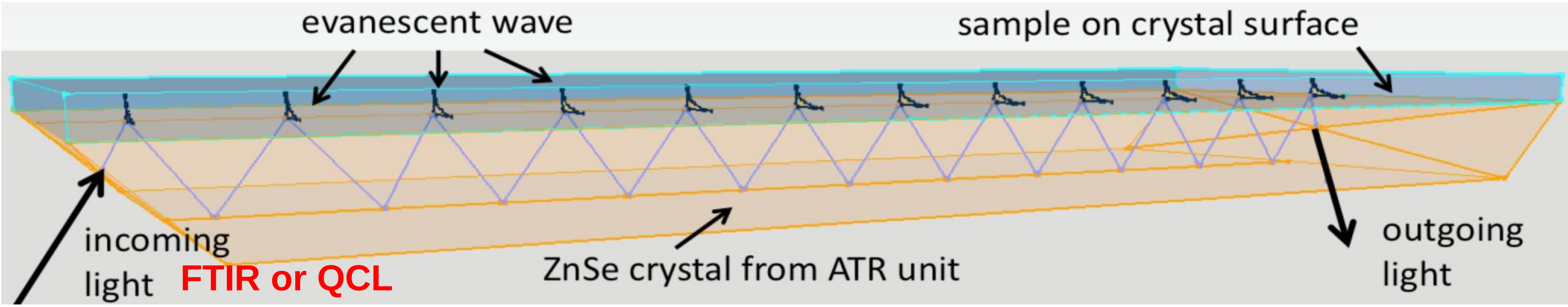
Positive: driver needs to provide a blood sample for lab analysis

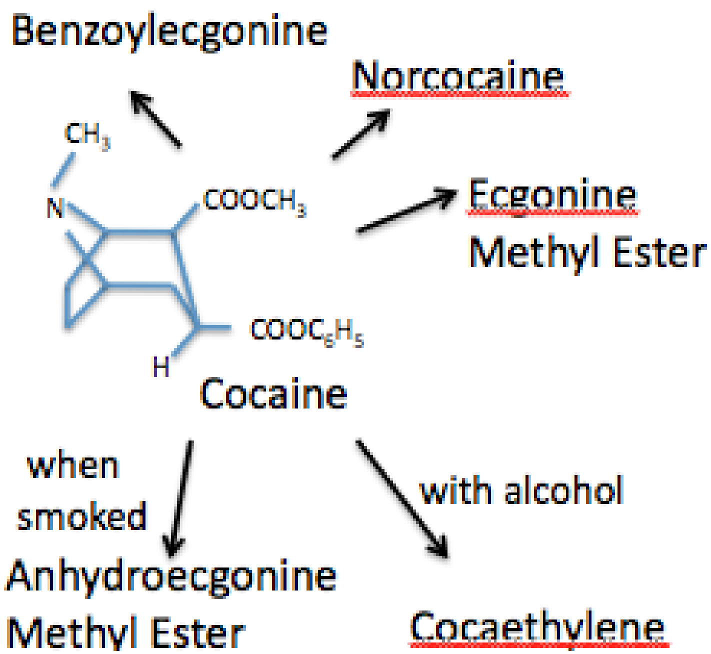
Problems

- relative high false positive/negative outcome (Wille et al. For. Sci. Int. 2010)
- No quantitative result on the street (no risk assessment)
- Second expensive analysis necessary

Optical detection method for cocaine and metabolites in body fluid

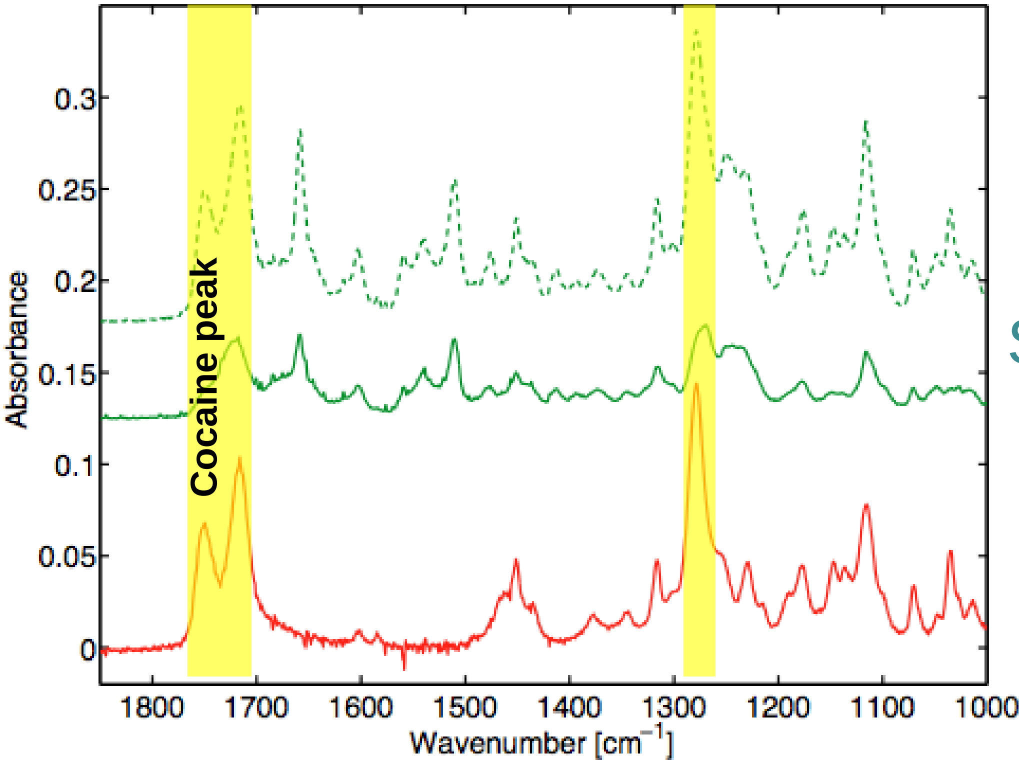
Attenuated Total Reflection (ATR) Spectroscopy





- Cocaine is metabolized in the body
- Cocaine and metabolites are in saliva
- Some metabolites are more toxic and longer active than cocaine itself
- Ratio between cocaine and metabolites varies => necessity to measure cocaine **and** metabolites for quantitative results

FTIR-ATR cocaine spectra, role of interfering compounds



Street cocaine

In TCE, dried

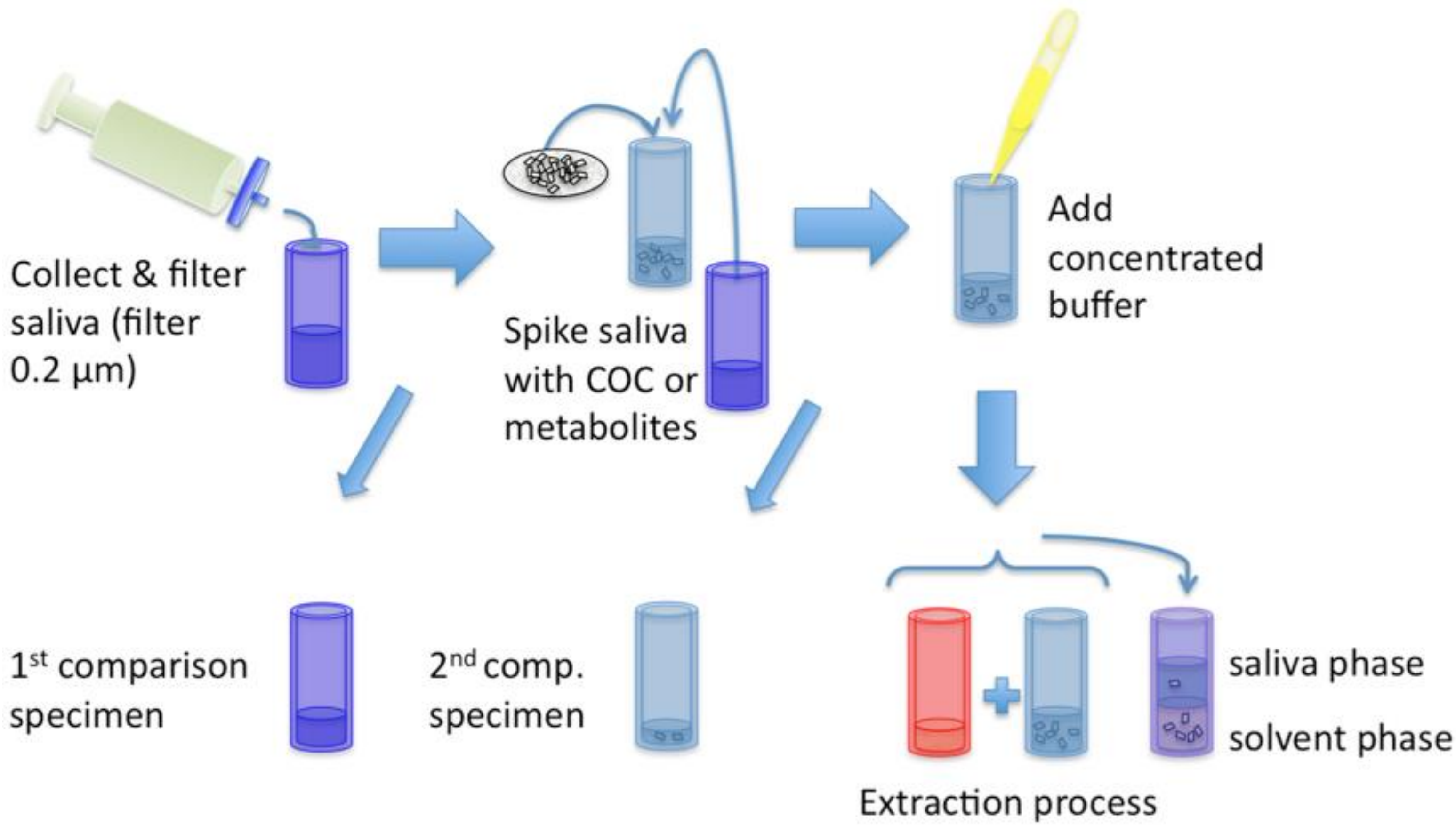
Pure cocaine

- **Cocaine absorption peaks: 1720 and 1750 cm^{-1}, around 1280 cm^{-1}**
- Lactose and Mannitol: 1200 to 1000 cm^{-1} ; Natron around 1350 cm^{-1}
- Caffeine between 1710 and 1200 cm^{-1}, 1st caffeine peak interferes with 2nd cocaine peak

Hans et al.: *Drug Testing & Analysis* **4**, 420429 (2012)

Taking of samples

Saliva yields high background signal ➡ Extraction into less absorbing solvent: tetrachloroethylene (TCE)

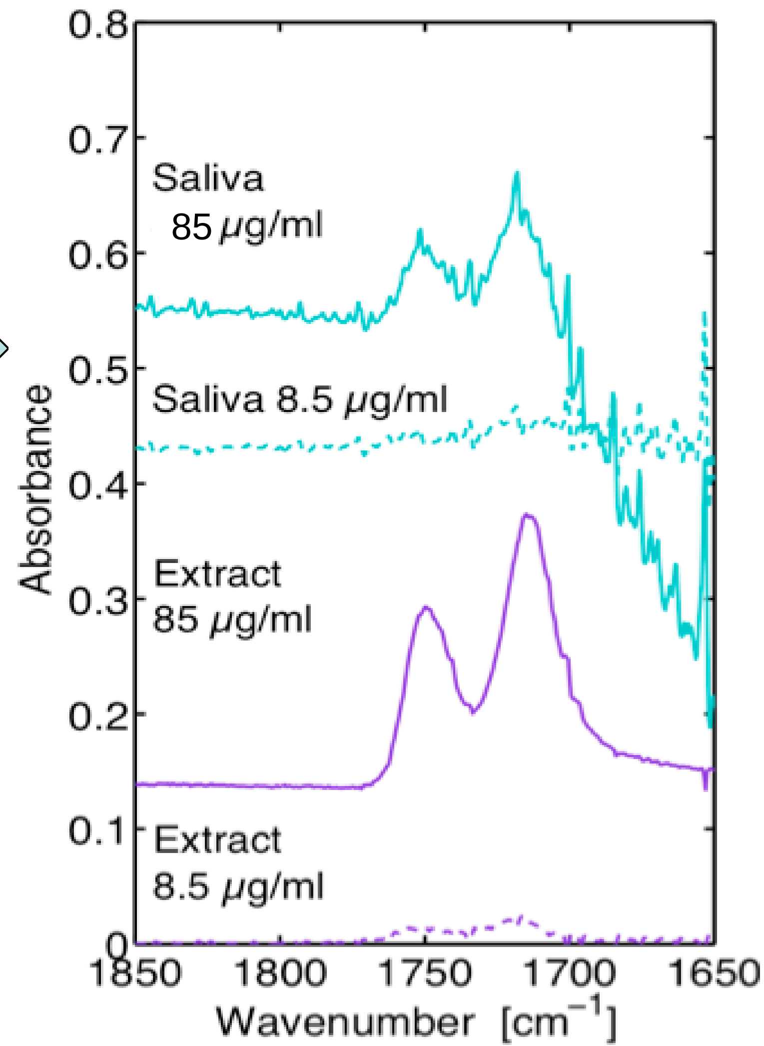


One-step extraction improvement of SNR

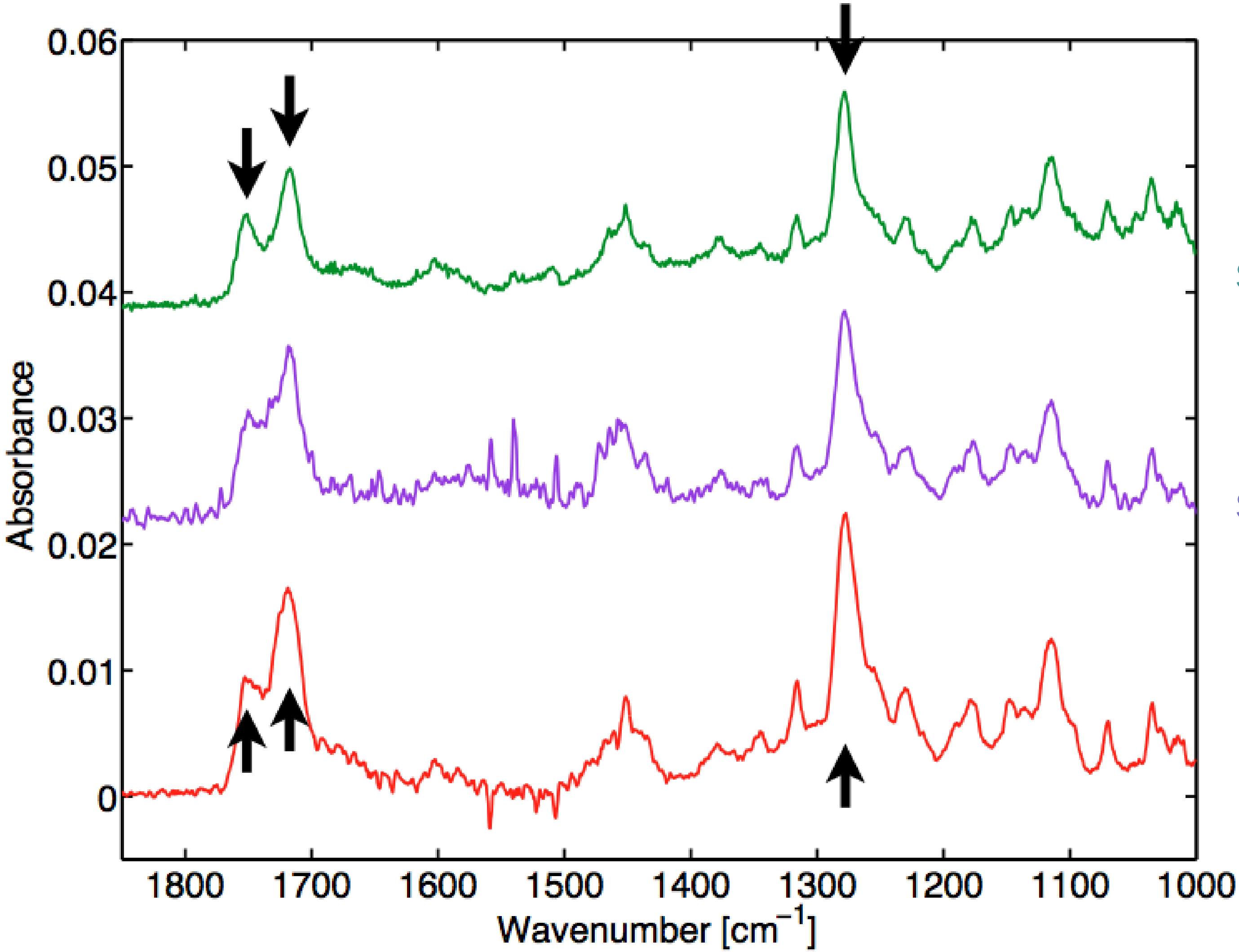
Extraction solvent:
Tetrachloroethylene (TCE)

Absorbance of filtered saliva subtracted from spiked saliva

Spectra of extracts (**no** subtraction necessary)



FTIR-ATR results: Extraction efficiency
Tetrachloroethylene (TCE) as extracting solvent

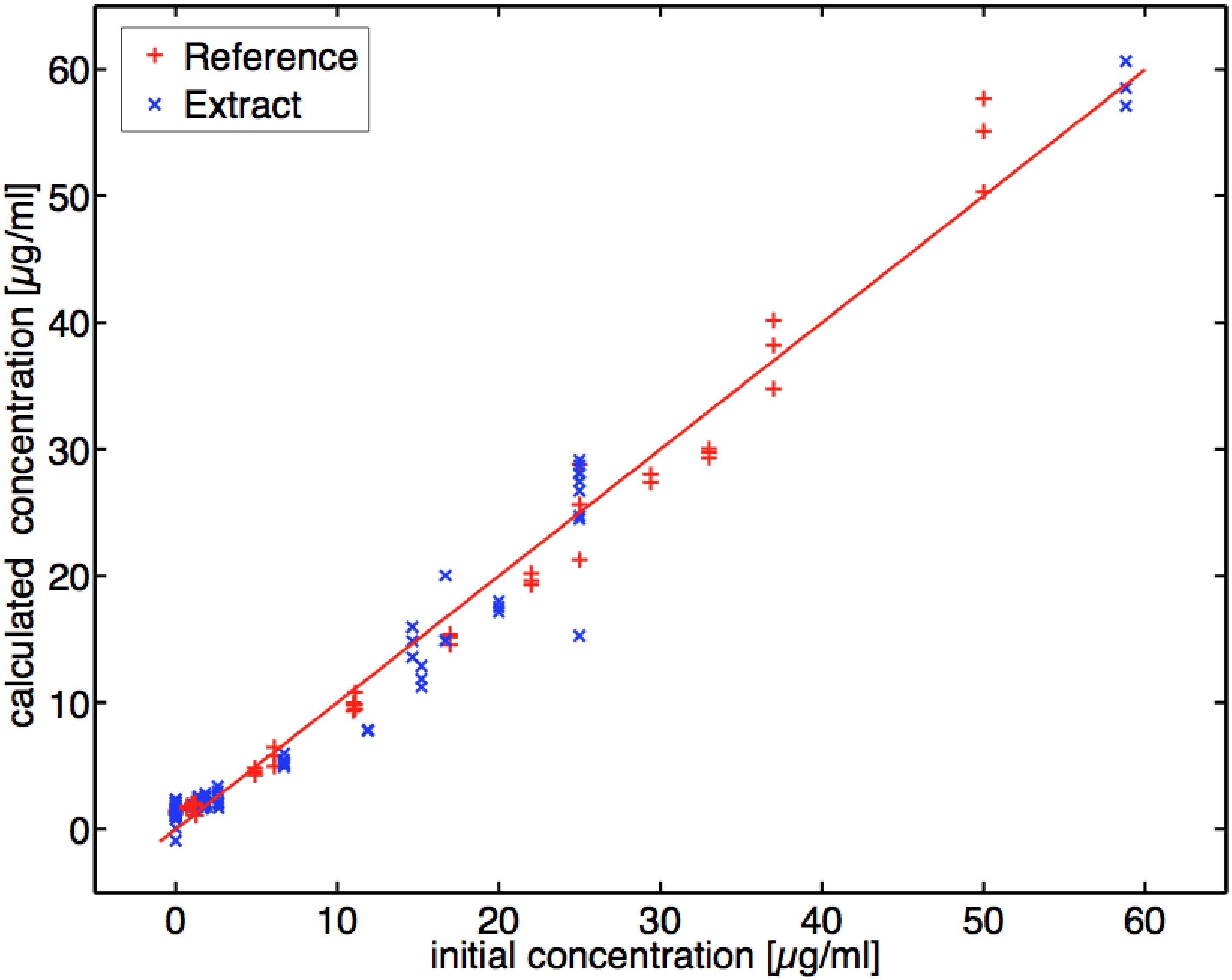


Extract from saliva spiked with street COC

Extract from saliva spiked with pure COC

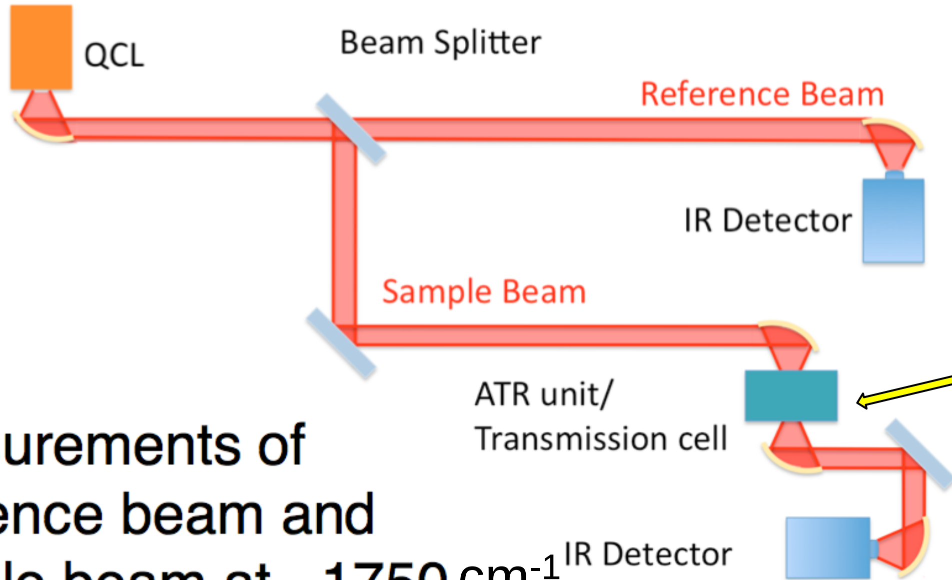
TCE directly spiked with COC as **reference**

Calculated vs initial COC concentration in saliva



- 6 test persons (age 27 – 64)
- 29 extractions
- Extraction efficiency: ≈100%
- Current LOD: ≈1 μg/ml (no preconcentration)

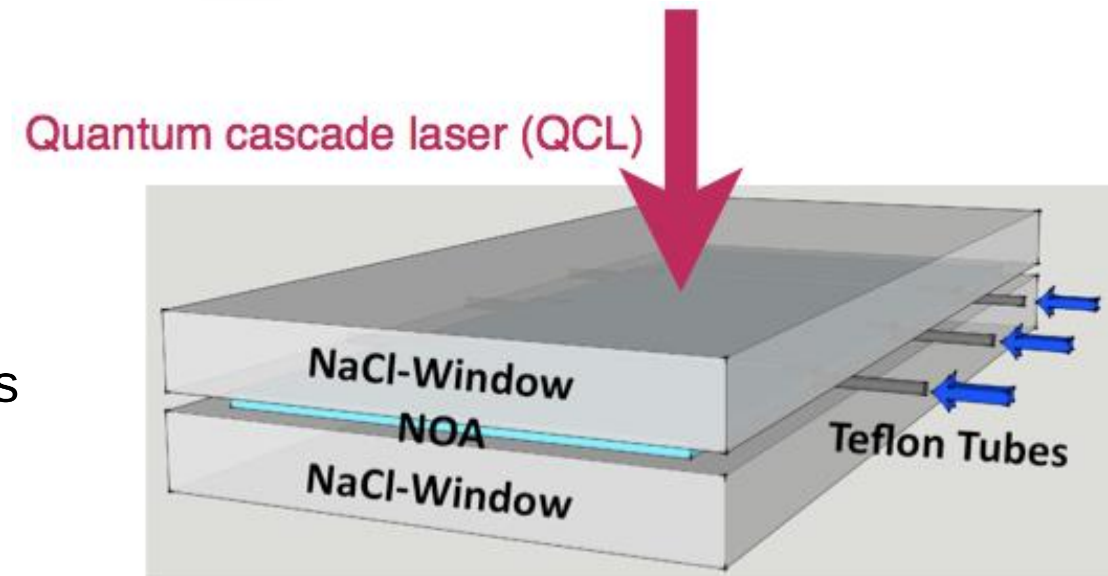
QCL transmission studies



QCl passes either trans-mission cell or ATR unit

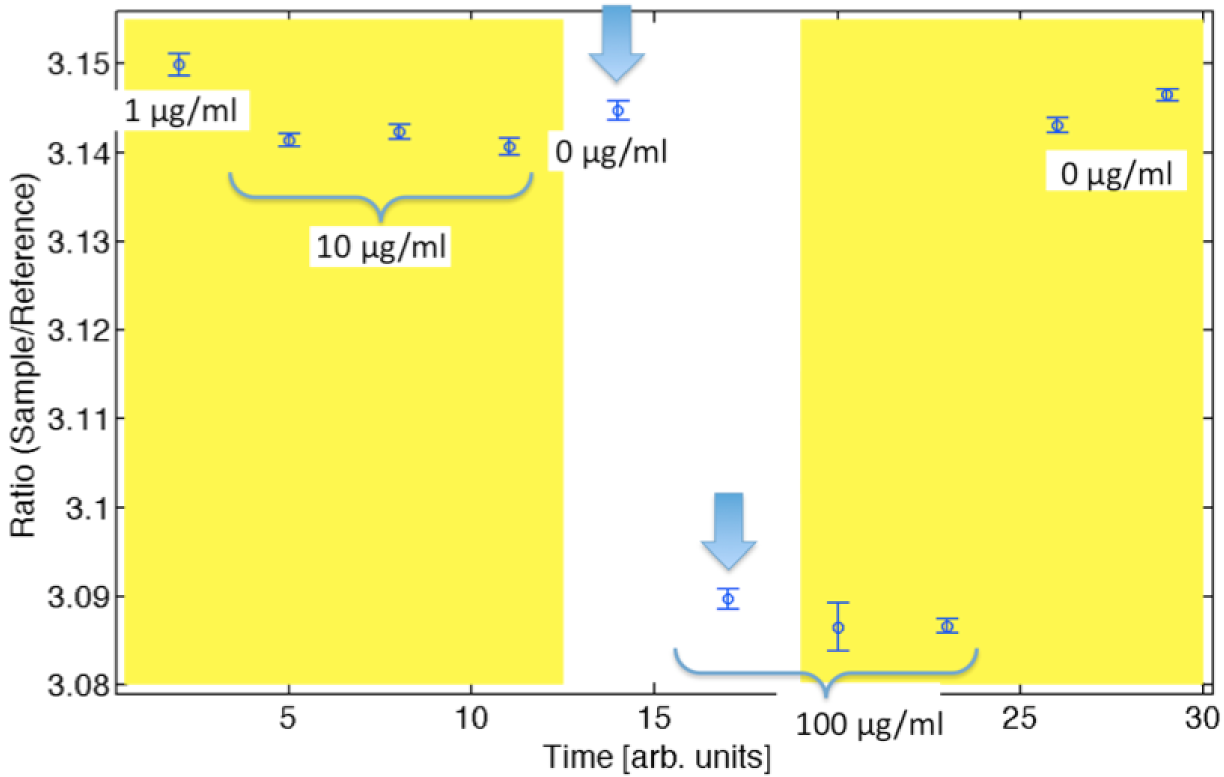
Measurements of reference beam and sample beam at ~1750 cm^{-1}

Transmission cell:
NaCl windows with NOA channels and Teflon tubes for sample inlet
Channel thickness: 1 mm



K. Hans et al.: *Proc. SPIE* **8591**, 85919F (2013)

QCL transmission measurements of reference samples



Stable conditions

Movements of transmission cell

LOD ≈ 10 µg/ml

Conclusions

- DFG for isotope ratio determination in breath
  Enhanced D/H ratio after ingestion of 5 ml D_2O (for weeks)
- DFG for analysis of surgical smoke
  quantitative *in vivo* studies, identification of several gases incl. anesthetic sevoflurane) at ppm level
- QCL and PAS for non-invasive glucose measurements
  in vitro: time-resolved diffusion of glucose in skin samples
  in vivo: promising results, stability, sensitivity ??
- FTIR-ATR and QCL detection of cocaine in saliva
  Extraction, μg/ml sensitivity with compact system
  Stability, sensitivity ??

Outlook

- New laser developments

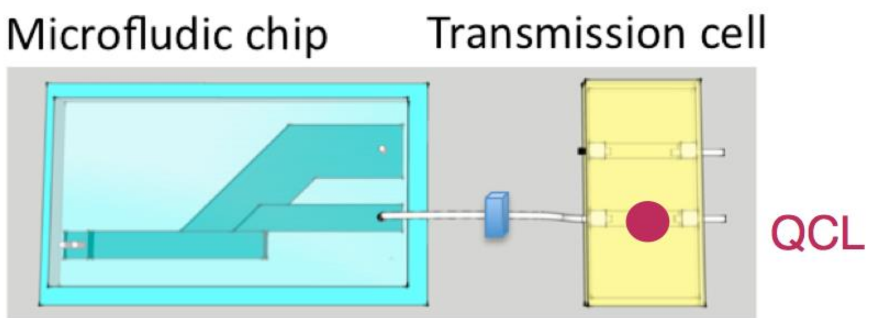
  ICLs, Pb salt VECSELs

  Frequency combs

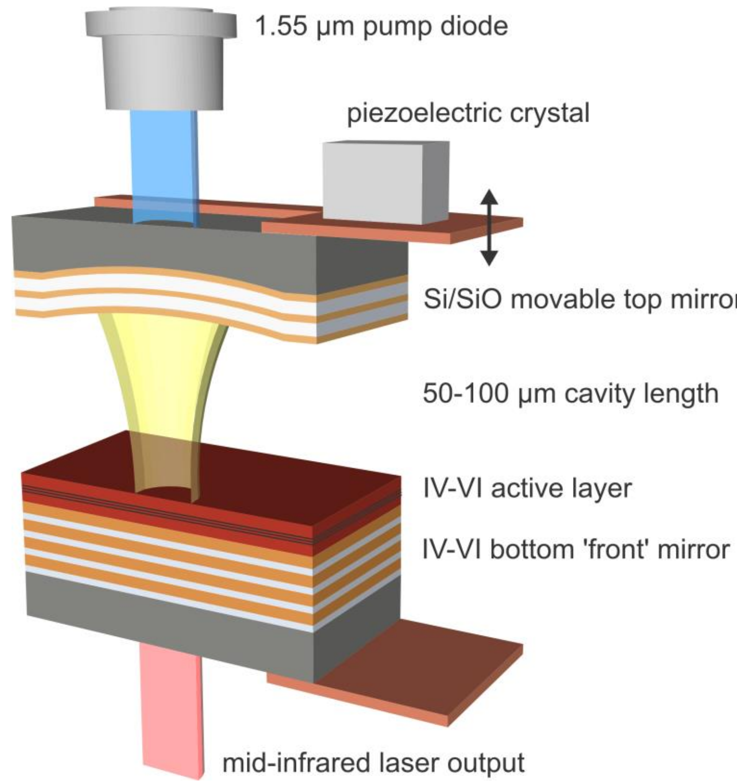
- Detection schemes

  Integrated devices

  Combination with microfluidics



Quantitative analysis for cocaine on a microfluidic chip



λ=3.4 μm, Tuning range: >70 cm^{-1}

Pulse power: 10 mW_p , Duty cycle: 0.5%

Collaboration: phocone, **Funding: KTI**

- New application areas

  Field & POC measurements, lab-on-a-chip

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Thank you for your attention

Assignment of the vibrational absorption bands

Table 6.1 – Assignment of the vibrational absorption bands of the measured FTIR transmission and ATR spectra of the investigated samples (i.e., keratinocyte solution Fig. 6.10, epidermal skin sample Fig. 6.12 and *in vivo* Fig. 6.17). The assignment is based on the references [138, 151, 192]. Used abbreviations: v = very, s = strong, m = medium, w = weak, sh = shoulder, br = broad, δ = deformation ρ = wagging, ν = stretch and ? = uncertain.

Frequency [cm^{-1}]	Assignment	Keratinocyte solution	Epidermal sample	*In vivo*
970	unknown	-	w	-
1035	ν(CC) skeletal *cis* conformation	-	sh	vw
1047	ν(C-OP)	sh,w	sh	vw
1077	ν(CC) skeletal *trans* conformation	?	?	w
1080	$\nu(PO_2^-)$ symmetric	m	m	w
1118	ν(CC) skeletal *trans* conformation	sh	sh	w
1170	ester ν(C-O) asymmetric	-	w	w
1240	$\nu(PO_2^-)$ asymmetric	ms	?	?
1245	ν(CN) amide III	?	m	m
1298	$\delta(CH_2)$	-	m	w
1342	$\rho(CH_2)$	-	vw	w
1401	$\delta[C(CH_3)_2]$ symmetric	m	m	m
1455	$\delta(CH_3)$ asymmetric	?	m	m
1545	δ(NH) and ν(CN) amide II	vs	vs	vs
1640	$\delta(H_2O)$ bending	vs	vs	vs
1650	ν(C=O) amide I	vs	vs	vs
1740	ν(C=O) lipid	-	w	vw
2125	$\delta + \nu_L(H_2)$ combination	-	m, br	w, br
2851	$\nu(CH_2)$ symmetric	w	m	m
2873	$\nu(CH_3)$ symmetric	w	w	vw
2920	$\nu(CH_2)$ asymmetric	m	m	m
2957	$\nu(CH_3)$ asymmetric	m	m	w