Quantum Cascade Laser based Breath Analysis of Human Diseases

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OUTLINE

FDA Workshop
Falls Church, VA
March 13, 2003

- Laser Spectroscopy for Breath Analysis
- Candidate Breath Biomarkers
- Measurement of Exhaled NO (Compact Gas Cells)
- Measurement of CO
- Measurement of OCS

Motivation: Laser Spectroscopy for Breath Analysis

Laser Absorption Spectroscopy (LAS)

- LAS provides rapid, sensitive and selective measurements of target gases
- LAS is capable of measuring multiple target gases with a single laser (important for standardizing exhaled breath analysis)
- LAS has been shown to be robust (e.g. – aircraft measurements)

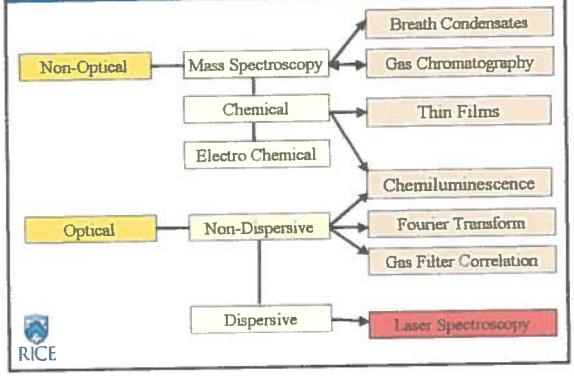
Target Gases – 1 (Immediate Candidate Markers)

Molecule	Formula	Trace Concentration in Breath (ppb)	Biological/Pathology Indication
Nitric Oxide	NO	6 - 100	Inflammatory and immune responses (e.g., asthma, COPD), vascular smooth muscle response
Carbon Monoxide	CO	400 - 3000	Hyperbilirubinemia, Smoking response, CO poisoning, vascular smooth muscle response, platelet aggregation
Hydrogen Peroxide	H_2O_2	1 - 5	Airway Inflammation, Oxidative stress
Carbonyl Sulfide	OCS	100 – 1000	Liver disease and acute allograft rejection in lung transplant recipients
Formaldehyde	HCHO	400 - 1500	Cancerous tumors, breast cancer

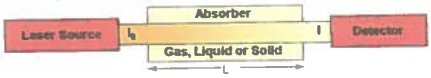
Target Gases -2

Molecule	Formula	Trace Concentration in Breath (ppb)	Biological/ Pathology Indication
Pentane	$CH_3(CH_2)_3CH_3$	4 - 20	Lipid peroxidation, oxidative stress associated with inflammatory diseases, immune responses, transplant rejection, breast and lung cancer
Ethane	C_2H_6	3 - 100	Lipid peroxidation and oxidative stress
Carbon Dioxide isotope ratio	$^{13}CO_2/^{12}CO_2$	$4 - 5 \times 10^{-3}$	Marker for Helicobacter pylori infection, GI and hepatic function, drug clearances rates
Methane	CH_4	1000 - 8000	Digestive function, colonic fermentation
Ammonia	NH_3	100 - 500	Hepatic encephalopathy, liver cirrhosis, fasting response
Acetone	CH_3COCH_3	1000 - 5000	Fasting response, diabetes mellitus response, ketosis

Existing Methods for Trace Gas Detection



Direct Laser Absorption Spectroscopy

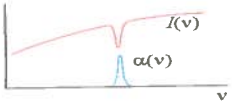


Beer-Lambert's Law of Linear Absorption

$I(\nu) = I_0 \cdot e^{-\alpha(\nu) \cdot P_a \cdot L}$

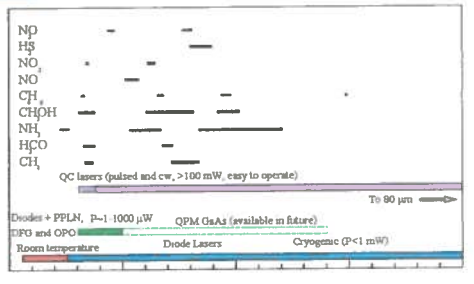
$\alpha(\nu)$ - absorption coefficient [cm^{-1} atm^{-1}]; L – path length [cm]
ν - frequency [cm^{-1}]; P_a - partial pressure [atm]

$\alpha(\nu) = C \cdot S(T) \cdot g(\nu - \nu_0)$



C - total number of molecules of absorbing gas/atm/cm^3 [molecule·cm^{-3}·atm^{-1}]
S - molecular line intensity [cm·molecule^{-1}]
g($\nu - \nu_0$) - normalized lineshape function [cm], (Gaussian, Lorentzian, Voigt)

Spectral Coverage by Diode & QC Lasers



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Key Characteristics of Quantum Cascade Lasers

- Laser wavelengths cover the entire range from 3.4 to 24 μm determined by layer thickness of same materials
- High power and single frequency (100 mW cw, 50 mW average, pulsed)
- Continuous tuning by temperature (~10 cm^{-1}) or current (~3 cm^{-1})
- Reliable, robust, and be operated with compact systems
- **Capable of near-room temperature operation**
  - Pulsed: up to +150°C (System Design)
  - CW: down to -63°C (Gas Cell Design)

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Exhaled Nitric Oxide

- **"Asthma is a chronic inflammatory disorder of the lower airways..."**
  - Working definition (NHLBI, 1995)
- eNO has a strong correlation with eosinophilic airway inflammation, considered a hallmark of bronchial asthma
- **Main Applications:**
  - Monitoring chronic airway inflammation by longitudinal eNO breath measurements
  - Monitor the effectiveness and compliance of anti-inflammatory therapies

Exhaled Nitric Oxide – Cont'd

- 30 to 80% of ex-asthmatics develop symptoms later in life and most have increased airway responsiveness to methacholine
- Suggested that ongoing airway inflammation is the principal cause of progressing airway abnormalities
- Airway symptoms correlate poorly with airway obstruction and indices of asthma severity
  - Adaptation of "perceptiveness" for bronchoconstriction
- van den Toorn et. al. recommended:

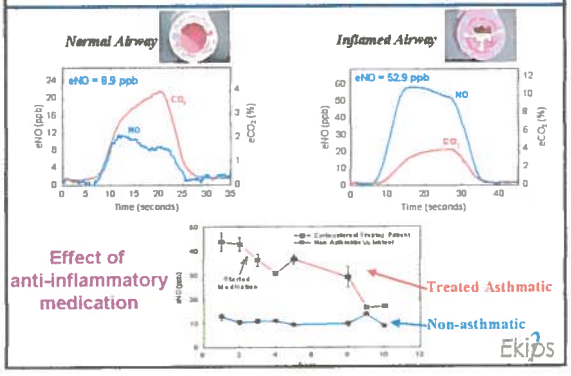
  "adolescents who seem to have outgrown asthma should be monitored for years after symptoms have disappeared, ... using non-invasive measurements of airway inflammation, such as eNO levels..."

  van den Toorn et. al., *Current Opinion in Pulmonary Med.*, 2003

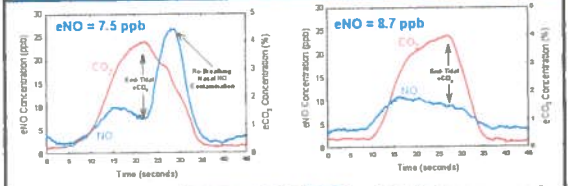
Point of Care
Exhaled Nitric Oxide Instrumentation

- **Chemiluminescence**
  - Used for over ten years to measure eNO in both children and adults
  - FDA approval efforts are ongoing (product code MXA)
  - Inter-study reproducibility issues
  - ATS has established recommendations for both online and offline measurements. Stresses the need for patient to maintain constant exhalation force
- **Laser Absorption Spectroscopy (LAS)**
  - Used to measure eNO in both children and adults
  - Demonstrated self-calibrated operation using exhaled CO_2 eliminating the need for the patient to maintain constant exhalation pressures: important when considering a pediatric patient

eNO Measurement Examples with Lead-Salt Diode Lasers



eCO_2 Helps to Standardize eNO Measurements



5-year old girl with severe asthma, treated with corticosteroids

Single Exhalation Against Resistance (10-20 sec)

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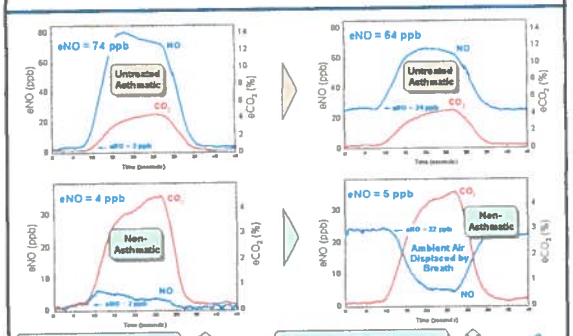
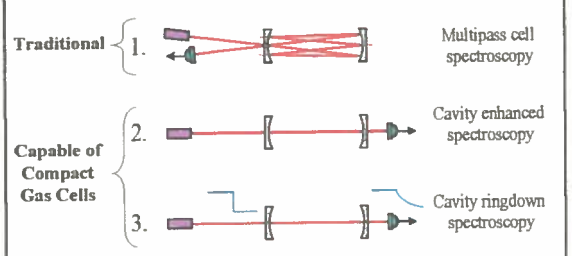
eNO and Atmospheric NO (aNO)



Ekips

Ultrasensitive Absorption Spectroscopy Techniques



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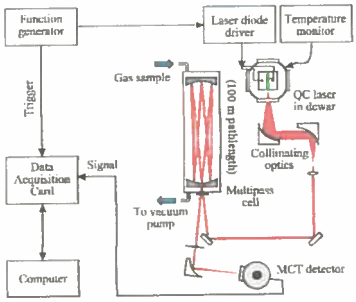
eNO Detection with a Multipass Cell and cw-Quantum Cascade Laser



- Conventional 100-meter optical Herriott cell

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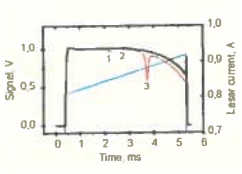
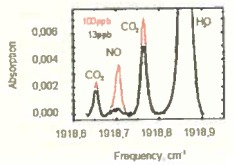
Breath Samples in the Multipass Cell



Transmitted intensity of pure Nitrogen (black) and a breath sample (red) at 50Torr and laser current (blue).
(1) H_2O @ 1918.860 cm^{-1}, (2) CO_2@ 1918.76 cm^{-1}, (3) CO_2@ 1918.585 cm^{-1}



Absorption of a breath sample from the nose (red) and the mouth (black) at 50Torr.

Detection limit 3 ppb

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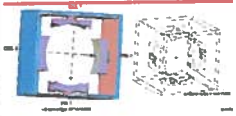
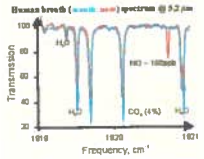
Cavity Enhanced Spectroscopy Breath Samples

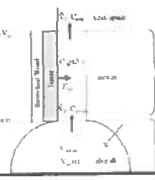
Novel Compact Gas Cell Design



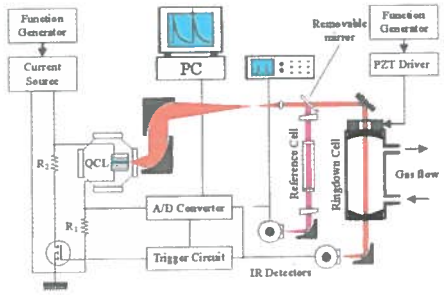
- Advanced optical gas cell design
- Capable of cell volumes < 10 cm^3
- Enables rapid measurements of eNO for flow independent eNO analysis
- Can distinguish between NO originating from airway walls or alveolar regions
- Useful in differentiating between asthma and alveolitis



Tsoukias et. al.



CRDS Based Gas Sensor



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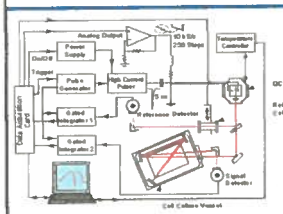
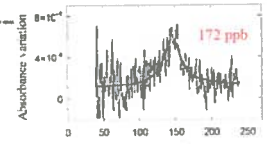
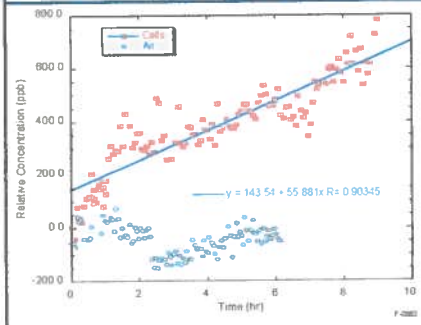
QC laser-based measurements of CO trace gas above cell cultures



- Measured CO production rates of viable cultures of vascular smooth muscle cells
- Achieved a detection limit of for CO of ~20 ppb



Measured CO production above cultured cells compared to control measurements without cell cultures



Important in understanding the kinetic or time dependent production of CO in heme catabolism

CO Production Rate ~ 44 ppb/h

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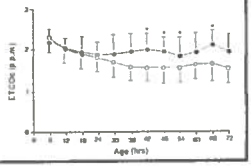
Exhaled Carbon Monoxide

- Exhaled CO is an FDA approved procedure for identification of carboxyhemoglobin (marketing towards neonatal bilirubin diagnostics)
- Natus Medical has received a 510(k) approval using electrochemical cell technology [#K974805]
- While useful in many cases, is not suitable for neonatal patients who:
  - are not in an open crib
  - are receiving oxygen via nasal cannula or hood
  - or, have high breath H_2 content

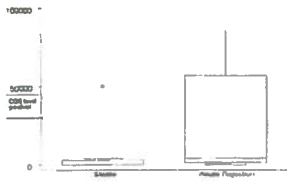
H. Okuyama, Pediatrics International, 43, 329-333 (2001)



Exhaled Carbonyl Sulfide

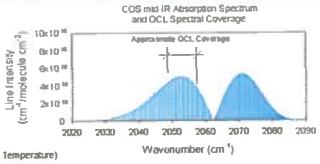
- Recent study by the T. H. Risby's group demonstrated elevated levels of OCS could have a diagnostic role in the detection of acute allograft rejection in lung transplant recipients
  - S. M. Studer, et. al., J. of Heart and Lung Transplantation, 20(11), 1158-66 (2001).

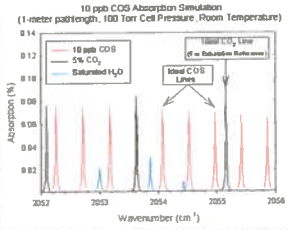
Measured with gas chromatography and flame photometric detection



OCS Mid-IR Absorption Spectrum

- OCS is among the strongest absorbers in the mid-IR





- Available CO2 absorption lines for assisting in the standardization of realtime expired breath analysis

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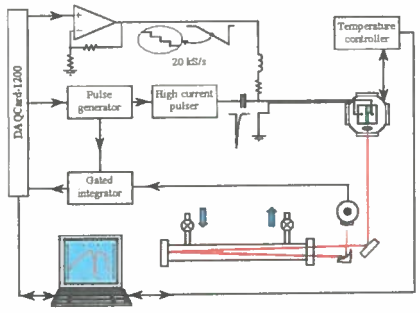
Pulsed QC Laser Based Gas Sensor



A. Kosterev et al, Applied Optics 41, 373 2002

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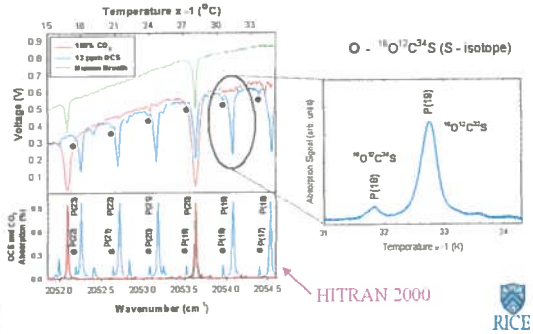
Acquired spectra of 13 ppm OCS with Isotopic Selectivity



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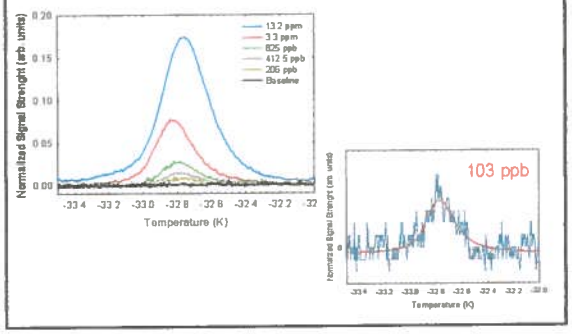
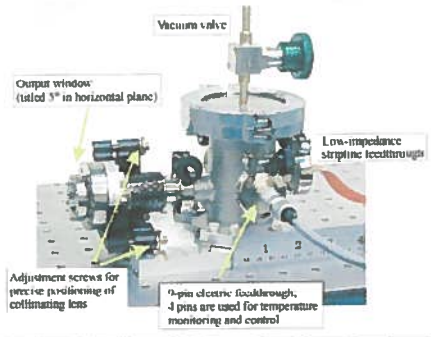
Preliminary OCS Concentration Measurements



TEC cooled QC Laser Housing



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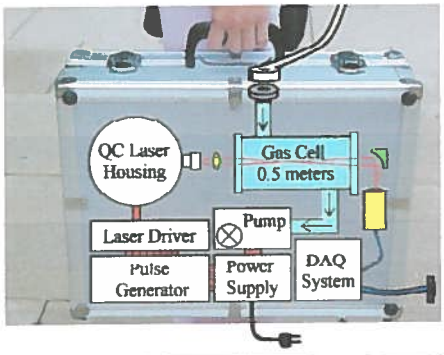
Schematic of a Portable LAS System



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Summary and Future Directions

- **Quantum Cascade Laser based Trace Gas Sensors**
  - Compact, tunable, and robust
  - High sensitivity (<10^{-9}) and selectivity (3 to 300 MHz)
  - Fast data acquisition and analysis
  - Detected trace gases: NH_3, CH_4, N_2O, CO_2, CO, NO, H_2O, OCS, C_2H_5OH and isotopic species
- **Applications in Exhaled Breath Analysis**
  - eNO: asthma, and alveolitis (e.g. interstitial pneumonia or idiopathic pulmonary fibrosis)
  - ET-CO: neonatal non-hemolytic hyperbilirubinemia
  - OCS: acute allograft rejection in lung transplant recipients
- **Future Directions**
  - Develop advanced compact optical gas cell for rapid eNO analysis
  - Begin clinical studies of exhaled OCS analysis
  - Place a robust and portable point-of-care mid-IR laser spectrometer in clinical setting

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Target Gases -3

Molecule	Formula	Trace Concentration in Breath (ppb)	Biological/ Pathology Indication
Hydrogen Sulfide	H_2S	10 - 30	Heart Diseases
Methyl mercaptan	CH_3SH	10 - 30	Oral infection, halitosis
Dimethyl sulfide	$C2H_3S$	2 - 20	Oral infection, halitosis
Isoprene	$CH_2 = C(CH_3)-CH=CH_2$	40 - 400	Cholesterol synthesis, acute myocardial infarction, ozone exposure, hemodialysis response, sleep/wakefulness monitoring
Acetylene	C_2H_2	Additive	Exogenous tracer to measure pulmonary function and cardiac output
Sulfur hexafluoride	SF_6	Additive	Exogenous tracer to measure pulmonary function

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