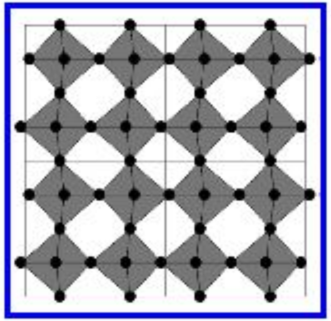
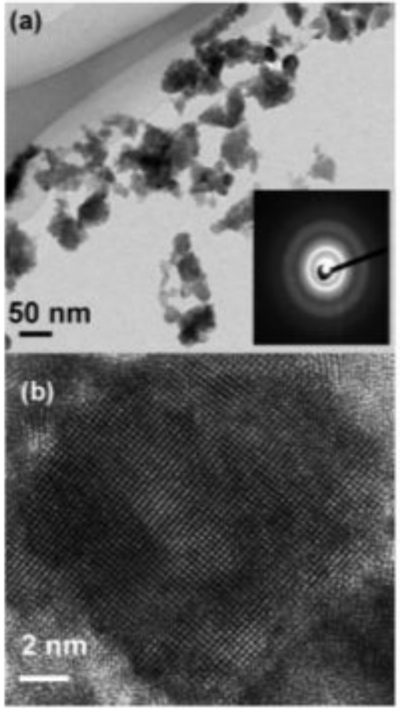
Asthma Monitoring Breathalyzer





NSF IIS #1231761

SHB: Type I (EXP): Personalized Asthma Monitor Detecting Nitric Oxide in Breath

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Program: Smart and Connected Health

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IIS Division of Information & Intelligent Systems

CSE Directorate for Computer & Information Science & Engineering

ABSTRACT This project explores the use of a gas selective resistive type sensing technology to detect and quantitate nitric oxide (NO) in exhaled breath for diagnostic purposes. The goal of the project is to develop a technique for personalized monitoring of the fraction of nitric oxide (FeNO) in exhaled breath, with the long term objective of prevention or control of airway diseases, such as asthma. FeNO is a known biomarker for measuring airway inflammation and the technology studied in this project provides an effective and practical means to quantitate NO levels in breath in a relatively simple and noninvasive way. This work involves the use of single crystal metal oxide nanowires that are expected to improve the detection threshold of NO-selective sensing nanoprobes by at least two orders of magnitude down to the few ppb level and below. A sensor microsystem is being developed that quantifies the gas sensor response to generate and display an accurate measure of the NO concentration in a single exhaled breath. The design of independent component analysis (ICA) algorithms, to enhance the gas discrimination and improve the robustness of the sensor response, is one of the objectives of this project. The implementation of the ICA algorithms and readout circuitry incorporating baseline tracking in mixed-signal VLSI will lead to a low-power autonomous system-on-chip solution for the measurement of gas concentrations from a handheld gas-measuring unit. The head space from cell cultures that have been stimulated to generate NO and CO are being used, along with breath-simulating samples, to assess the performance and reliability of the NO-breathalyzer. The same studies help to understand the biochemistry of NO production in response to inflammation. The expected outcome of this work is a smart health breath analysis tool that will empower the individual who may be susceptible to airway diseases to stay healthy and that provides a means for self-monitoring of early signs of illness in the home, instead of the hospital setting in which monitoring would require the assistance of health care professionals. The new tools being developed for personalized diagnostics should be easily employed by the lay public to promote their health and well-being. Furthermore, the device will be especially suitable for use by a wide range of compromised individuals, such as the very elderly, young children and otherwise incapacitated patients. This project will engage students, including underrepresented groups in research activities in an interdisciplinary field spanning nanomaterials, sensor nanotechnology, microelectronic device fabrication and diagnostic instrumentation, biophysics and biochemistry, and ultimately nanomedicine. Interactions with National Laboratories and the medical diagnostics industry are anticipated. These interactions are expected to lead to the translation of the technology embodied in the NO-breathalyzer resulting from this work in the laboratory to the marketplace. The results of this work will be disseminated through publications, presentations, outreach events and multimedia products to be posted on the project web site.

Year 1 (2012-2013)

- http://commcgi.cc.stonybrook.edu/am2/publish/General_University_News_2/SBU_Researchers_Win_NSF_Award_for_Asthma_Breath_Analyzer.shtml

- http://www.insidescience.org/content/cellphones-detecting-asthma/980

SHB: Type I (EXP): Personalized Asthma Monitor Detecting Nitric Oxide in Breath

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Abstract

Nitric oxide is a well-established biomarker for airway disease monitoring and there are set guidelines regarding the concentration of this gaseous biomarker in human exhale for various medical conditions. Based on these guidelines, our project aims at developing handheld and inexpensive single breath exhale monitors (NO breathalyzers) that will assist with early asthma diagnosis and easy monitoring of the disease. The approach followed is based on selective chemosensing using resistive sensors with polymorphic metal oxide sensing elements. We have succeeded thus far in processing single crystal nanowires of the □-MoO_3 and □-WO_3 polymorphs, both materials being NO selective sensing probes. Recent progress is shown in sensing trace NO amounts in breath simulated environments and also in developing numerical devices for breath collection, monitoring and display of the NO concentration in a single exhale.

BACKGROUND

- A chemo-resistive gas sensor is a device which reacts with its surrounding gas and converts this reaction into a change of its electrical resistance in a distinctive manner
- Polymorph control in nanostructured metal oxides enables them to become gas-selective chemo-resistors
- Our nanostructured sensors have specific affinity to the targeted gaseous biomarker

Crystallo- Chemical Approach to Gas – Metal Oxide Interactions

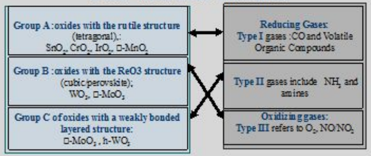
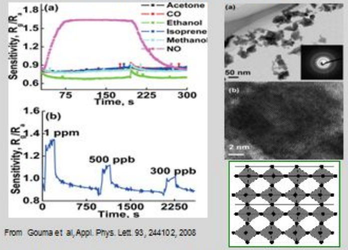


Table1: Selectivity of certain oxide structural groups to classes of gases [1-2]

Results from earlier studies by the PI

NO detecting selective nanosensor based on nanostructured thin films



From Gouma et al, Appl. Phys. Lett. 93, 244102, 2008

Figure 1: Earlier sensing results for NO sensing by thin films of □-WO_3 [3]

Patents granted:

- US/Patent No **7,017,389** issued on 3/28/2006, "Sensors Including Metal Oxides Selective for Specific Gases and Methods for Preparing Same", by **P.I. Gouma**
- US/Patent No **7,981,215** issued on July 19, 2011, "Electrospun Single Crystal MoO_3 nanowires for bio-chem sensing probes", by **P.I. Gouma**, A. S. Haynes, and K. Kalyanasundaram

Detecting NO biomarkers

Disease Marker	Typical Concentration In human breath	Our NO detecting Sensors
Asthma	Nasal Few ppm	• □-phase MoO_3 • Sol-gel • Nanowires
Oxidative stress Lung diseases	Breath Low ppb	• □phase WO_3 • Sol-gel • Nanowires
See ATS Clinical Practice Guidelines [4]		

Table 2: NO is a biomarker for airway diseases in a given concentration range

- Key biomarker: **NO** in breath
- Measuring FENO measures airway inflammation [4]
- NO is detectable in exhaled air in significant amounts: from 0.2–1 ppm in the upper respiratory tract; and 1–30 ppm at the nasal level
- Both the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have published guidelines for the measurement of FENO]

MATERIALS & METHODS

Novel Sensing Materials

β-phase MoO_3 γ-phase WO_3

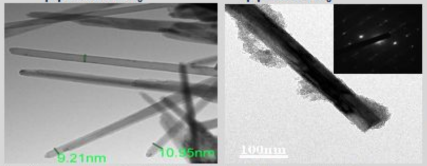


Figure2: Single crystal nanowires of the ReO_3 structural group that are expected to detect NO with high specificity:(Left) MoO_3 and (Right) WO_3

References

1. P. Gouma, Nanomaterials for Chemical Sensors and Biotechnology, Pan Stanford Publishing, 2009.
2. a. P. I. Gouma, "Nanostructured Polymorphic Oxides for Advanced Chemosensors", Rev.Adv. Mater. Sci., 5, pp. 123-138, 2003; b."Polymorphism in nanocrystalline binary metal oxides", S. Sood, P. Gouma, Nanomaterials and Energy, 2(NME2), 1-15(2013).
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4. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR, "An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications.", Am J Respi Crit Care Med 184, pp. 602-615, 2011.
5. P. Gouma, K. Kalyanasundaram, X. Yun, M. Stanacevic and L. Wang, "Chemical sensor and breath analyzer for ammonia detection in exhaled human breath", IEEE Sensors, Special issue on Breath Analysis, 10 (1), pp. 49-53, 2010.
6. P. Gouma, A. K. Prasad and M. Stanacevic, "Selective nanosensor device for exhaled breath analysis", J. Breath Res., 5, 037110, 2011.
7. P. Gouma, "Interview: Revolutionizing personalized medicine with nanosensor technology", Person. Med. 8(1), pp. 15-16, 2011.

Novel Sensor Design and Testing

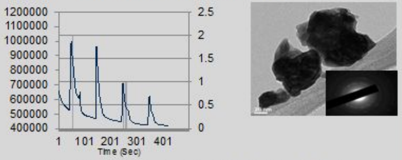

Figure 3: (Left)Sensing data for γ-WO3 nanosensors tested for 1ppm and 2ppm NO gas; (Right): Morphology of the grain structure of the sensing elements.

Binary (On/Off) handheld breath analyzer

HANDHELD DEVICE PROTOTYPE

Our innovation lies with empowering the individual to acquire affordable, non-invasive medical diagnostic tools for home use

Figure 4: Binary breath analyzer [6]

DIMENSIONS

- Teflon chamber of binary prototype: 15cm (L) x 7.5cm (W separates sensor from electronics and the environment
- Channel with mouthpiece controls breath flow to sensor

From P.I. Gouma et al, IEEE Sensors, 10 (1), pp. 49-53, 2010

Numerical Breath Analyzer

Figure 5: (Left)Numerical breath analyzer; (Right) Sensor chip that is the brain of the numerical analyzer [7].

SPECIFICATIONS

- Single breath, portable, handheld, numerical
- 3 sensor device, battery operated; 1 year lifetime
- One sensor is for CO_2 detection; used for standardization/calibration purposes
- Stand alone device- Instant digital readout of gas concentration; no need for computer or pattern recognition software

[from Gouma et al, J. Breath Res. 5 , 2011

A LOW-POWER WIDE-DYNAMIC RANGE READOUT IC FOR BREATH ANALYZER SYSTEM

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Background and Introduction

Task: detection and discrimination of signaling metabolites (disease markers) in a complex fluid, as is exhaled breath, and their measurement in trace concentrations.

Measuring the low concentrations of analyte molecules in breath is a major challenge, along with the specificity to a given gaseous chemical.

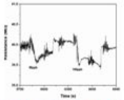
Objective: to develop a stand-alone selective chemical detection sensor array micro-system that would operate as a robust and reliable personal breath analyzer.

By controlling the microstructure of nanocrystalline metal oxide films of the sensor so as to employ oxide polymorph phases that are sensitive to only a specific class of gaseous analytes or even be specific to a single species have been derived. Selective NO, ammonia, and acetone breath analyzer prototypes have been produced.

top view

side view

Sensor and heater assembly.

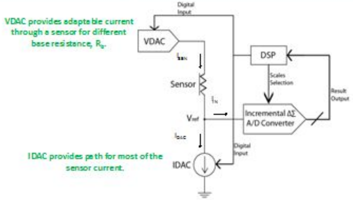


Sensing response of M_xO_y sensor to NH_3 (50ppb and 100ppb).

Readout techniques are required to address inherent properties of the sensor, particularly:

- their large baseline resistance that can be few orders of magnitude higher than the actual sensor response
- large variability of base resistance across sensors
- the drift in base resistance over time at a different rate across sensors.

Readout System Architecture



Block diagram of the readout circuit

Sensor baseline resistance range: 1kΩ ~ 100MΩ

0.05% ~ 10% of sensor resistance change detectable

Total dynamic range: 166dB

VDAC and IDAC compensate for the wide variation range of the baseline resistance. ADC tracks the change in sensor resistance with a change in gas concentration. As most of the sensor current from the baseline resistance is compensated by IDAC, the required resolution of the current ADC is significantly reduced. By adjusting the digital input value of the two D/A converters, the interface circuit can be both power effective and highly accurate.

Circuit Implementation

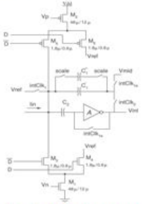
A. Incremental ΔΣ ADC

The delta-sigma ADC used in the system comprises a current integrator, comparator and switched-current single-bit D/A converter.

The ADC has two scales, selected by C_1' and C_1'' according to the input current range.

The ADC achieves 13 bit resolution.

The sampling frequency of the ADC is set to 100 Hz, which corresponds to the low-changing gas detection environment.



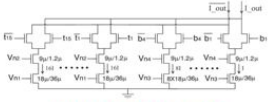
Schematic of delta-sigma current integrator

B. Segmented current-steering DAC

The 8 bit current mode D/A converter of the system is implemented using segmented current-steering structure.

4 more-significant bits use thermometer code to guaranteed monotonicity, good differential nonlinearity and very low glitches.

4 less-significant bits use binary-weighted to consume less area and reduce power.



Schematic of current mode D/A converter

System Operation

In order to measure the sensor resistance, the gas-sensing system has two phases of operation.

Calibration phase:

Digital logic decides the digital inputs of the two DACs according to a predesigned matching algorithm to guarantee the input current of ADC is smaller than the LSB of IDAC and eliminate the measurement error caused by the baseline resistor deviation as well.

Measurement phase:

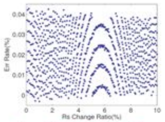
The sensor resistance changes due to the concentration of the gas of interest and all of the current variation is measured by the ADC.

This circuit purely measures the current difference between the two stages, thus it is much easier to achieve high precision with a less complicated ADC compared with a single ADC design.

Simulation Results

A. System Sensitivity Simulation

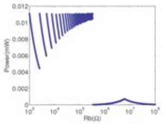
Sensor resistance change ratio: 0.05% ~ 10%
Baseline resistance :100MΩ
Smallest input current
Error rate < 0.045%



Error of ADC as a function of the sensor resistance changes

B. Power Consumption Simulation

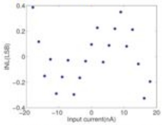
Sensor resistance change ratio: 10%
Baseline resistance: 1KΩ~100MΩ
Largest current variation
Maximum Power: 113μW



Power consumption of the readout system as the function of the baseline resistance

C. ADC Simulation

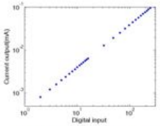
Input current range: ±18nA
I_{lsb} = 9pA
Integral Non-linearity < ±0.4LSB



Integral nonlinearity (INL) of current ΔΣ ADC

D. DAC Simulation

Input range: 100uA
I_{lsb} = 400nA



Current mode D/A converter output linearity

Conclusion

The designed interface circuit compensates for the variation in the baseline resistance of the gas sensor and guarantees error rate less than 0.045% at the power consumption on the order of 100μW.

The implemented readout ASIC that interfaces an array of nanosensors will be integrated in a nitric oxide (NO) breath analyzer for monitoring and managing airway diseases, such as asthma.

The proposed breath analyzer technology may also be used as a coarse diagnostic tool to enable an early detection and to direct more complex diagnostic tools where to focus attention.

References

Y. Lin, P. Gouma and M. Stanaćević, "A Low-Power Wide-Dynamic-Range Readout IC for Breath Analyzer System", Proc. IEEE Int. Symp. Circuits and Systems (ISCAS'2013), Beijing, China, May 2013.

P. Gouma, K. Kalyanasundaram, X. Yun, M. Stanaćević and L. Wang, "Nanosensor and Breath Analyzer for Ammonia Detection in Exhaled Human Breath", *IEEE Sensor Journal*, vol. 10(1), pp. 49 53, 2010.

L. Wang, K. Kalyanasundaram, M. Stanaćević and P. Gouma, "Nanosensor Device for Breath Acetone Detection", *Sensors Letters*, vol. 8, 2010.

Acknowledgment

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A Human Cell-Based Model of Airway NO Production in Response to Inflammatory Stimuli: A Source of NO in Exhaled Breath to be Detected by a Hand-Held Device

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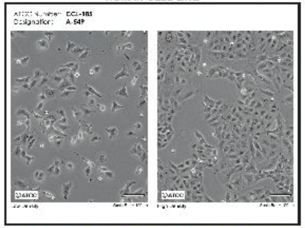
RATIONALE

The ultimate goal of our project funded by the Smart Health and Wellbeing program of the National Science Foundation is to develop a hand-held device to detect nitric oxide (NO) in exhaled breath as a quantitative indicator of inflammation in the lungs and airways, as well as in more distant sites throughout the body. NO is formed in human airway cells in response to direct stimulation by foreign particulates as well as the presence of cytokines released from remote tissues that reach the airway cells through the bloodstream. The presence of NO that diffuses out of stimulated airway cells has been used as a surrogate biomarker of asthma. In this exploratory project, we are using human airway cells in culture to generate NO rather than entering directly into clinical trials with human subjects. We maintain a human alveolar epithelial cell line in chambers from which the atmosphere above the cells can be collected for injection into our hand-held NO detector. The agents we are employing to trigger NO release from the airway cells have been implicated in asthma attacks in patients, providing a rationale for our model.

MATERIALS AND METHODS

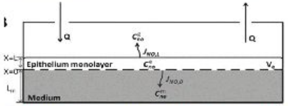
HUMAN CELL LINE



The A549 cell line was established by George Todaro's laboratory from a patient with lung cancer in 1973 and characterized as having characteristics of Type II alveolar epithelial cells (Lieber et al. 1976).

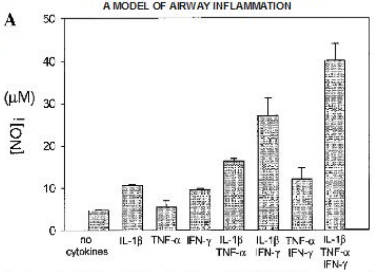
We culture A549 cells obtained from the American Type Culture Collection as monolayers in Ham's F-12K medium in the presence of 10% fetal bovine serum.

CULTURE METHOD



To approximate the air-liquid interface which lines the surface of human airways in vivo, we plate the cells onto enclosed polystyrene rectangular trays (335 mm x 205 mm; 632 cm²) with ports to collect either the culture medium or the atmosphere above the monolayers (Nunc Cell Factory). A similar design has been employed by the laboratory of S.C. George at U.C. Irvine (Kwon et al., 2001; Jiang and George, 2011) to detect release of NO from A549 cells in response to the inflammatory cytokines Interleukin 1-β (IL-1β), Interferon-γ (IFN-γ, and Tumor Necrosis Factor-α (TNF-α).

STIMULATION OF A549 CELLS WITH CYTOKINES
A MODEL OF AIRWAY INFLAMMATION

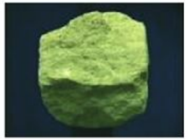


As previously reported by George's laboratory (Kwon et al., 2001), A549 cells release levels of NO in response to a cocktail of inflammatory cytokines that are about five times those released by cells cultured in cytokine-free medium. Circulating levels of these cytokines are also known to be elevated in patients experiencing an asthmatic attack or suffering from exacerbations of dieases marked by systemic inflammation. If these patients breathe into a mylar bag, the expired air can be subsequently anlyzed for NO using laboratory instruments. A more convenient strategy to facilitate increased patient compliance and better data collection is based on the hand-held device we are now developing, which can be employed to monitor such patients at home or workplace.

STIMULATION OF A549 CELLS WITH MINERAL POWDERS
A MODEL OF ENVIRONMENTAL AND OCCUPATIONAL ASTHMA

FRAMBOIDAL PYRITE (FeS)

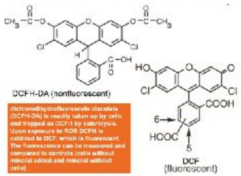
OLIVINE ($Mg_2Fe_2SiO_4$)



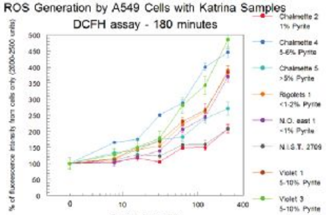
Airway epithelial cells can also be stimulated by direct exposure to dusts with different mineral compositions. Iron-containing minerals, such as pyrite and olivine, are present in dusts generated by mining operations, floods that leave quantities of mineral-rich mud and silt, and debris arising from construction or demolition sites. These dusts have been reported to trigger exacerbations of asthma in populations exposed to the mineral particulates, as has occurred in New Orleans after Hurricane Katrina and in New York City after the World Trade Center collapse.

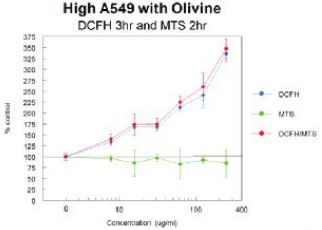
We have modeled the environmental exposure of airway epithelium to mineral dusts by incubating A549 cells with finely ground samples of pyrite and olivine. Before quantitating release of NO by these cells, we demonstrated that the mineral dusts induce other biomarkers of an inflammatory response. A reliable biomarker of inflammatory activation of A549 cells is the generation of Reactive Oxygen Species (ROS) that typically accompanies NO production.

STIMULATION OF A549 CELLS BY MINERALS



We detect the generation of ROS in A549 cells exposed to mineral particulates using the fluorogenic dye, dichlorodhydrofluorescein.





We will use these same incubation conditions to expose A549 cells cultured in Nunc Cell Factory units to pyrite and olivine and collect the headspace atmosphere to be injected into our hand-held NO detection device.

REFERENCES

Lieber, M., B. Smith, A. Szakal, W. Nelson-Rees, and G. Todaro (1976) A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int. J. Cancer 17:62-70.

S. Kwon, R.L. Newcomb, and S.C. George (2001) Mechanisms of synergistic cytokine-induced nitric oxide production in human alveolar cells. Nitric Oxide 5:534-546.

Jiang, J., and S.C. George (2011) Modeling gas phase nitric oxide release in lung epithelial cells. Nitric Oxide 25:275-281.

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Year 2 (2013-2014)

Intellectual Property related to the scope of the project:

Selective Nanoprobe for Olfactory Medicine:

- *US patent 8485983*, issued 07/16/2013
Inventors P.I. Gouma and S.R. Simon
http://www.google.com/patents/US8485983

- *US patent 8758261*, issued 06/24/2014
Inventors P.I. Gouma and S.R. Simon
http://www.google.com/patents/US8758261

Targeted Application: Asthma Monitoring

Application:

- **Nitric Oxide (NO) breathalyzer for monitoring of airway diseases** (such as asthma)
- consumer product, personalized monitoring of **fractional nitric oxide concentration (FENO)** in breath, home use
- Competition: three FDA approved devices for hospital use only costing $$$s

Biomarkers:

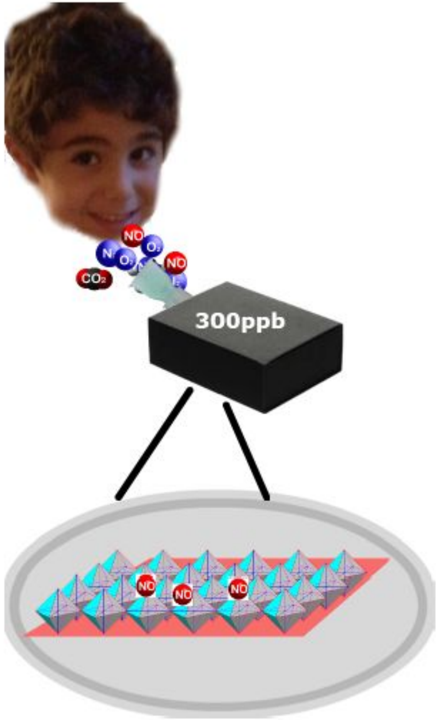
• Key biomarker: **NO** in breath
• measuring FENO measures airway inflammation ****
• NO is detectable in exhaled air in significant amounts: from 0.2–1 ppm in the upper respiratory tract; and 1–30 ppm at the nasal level
• Both the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have published guidelines for the measurement of FENO

Validation:

- Sensor tests measuring NO concentrations ranging from 300ppb to 1ppm have been carried out using synthetic air mixtures

****R.A. Dweik et al, "Am. J. Respir. Crit. Care Med, 184, pp. 602-615, 2011.

Personalized Asthma Monitor



Outcome: Scientists in New York develop a hand-held device for measuring nitric oxide concentrations in a single exhaled breath that could be used effectively by families in the home at least once each day without the need for constant supervision by medical professionals.

Impact/Benefits: The data to be generated by the NO-breathalyzer may potentially refine approaches for medical management of children with asthma and to adjust their medications before they have an exacerbation.

Explanation: Diagnosis and management of asthma in children is an especially challenging problem for health care delivery teams. Nanowire NO-selective gas sensors and advanced micro-chip technology detects gas molecules at part per billion concentration, in a single breath, and such devices can be used frequently by young children in a home setting so that their asthma does not compromise their social and educational progress, and remains under control at all times.