PHOTONICS APPLIED: BIOPHOTONICS: Breath analysis research approaches clinical practicality

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Clinicians anticipate pra[illegible]pact photonic test instrumentation to easily identify the several hundred different molecular species in ex[illegible]th that can indicate disease.

Imagine a time when a t[illegible]doctor's office to evaluate your ailment doesn't involve a painful blood draw or the hassle of a urine samp[illegible]entails a simple request to blow into a tube. Or perhaps a trip to the doctor isn't required at all: What if an evaluation could be conducted remotely by blowing into an accessory on your cell phone? This scenario is becoming a reality as the burgeoning field of breath analysis provides new and, perhaps most important, noninvasive methods to detect abnormal function within the body. Many of the leading techniques involve optical and laser-based approache[illegible]providing new capabilities to bring breath analysis into a clinical setting.

The notion of detecting c[illegible]ates in exhaled breath has existed for almost 2500 years. Hippocrates described specific correlations between breath aroma and disease in one of his many medical treatises; in 1784, Lavoisier and Laplace showed that respiration consumes oxygen and eliminates carbon dioxide; in 1897, Nebelthau showed that diabetics exhale acetone in their breath; and in 1971, Pauling used gas chromatography (GC) to detect over 250 compounds in exhaled human breath.

Since that time, advances in mass spectrometry (MS), combined GC-MS techniques, chemiluminescence sensors, electrochemical sensors, and infrared (IR) spectroscopy have allowed researchers to identify more than 5000 unique substances in exhaled breath. These substances include elemental gases such as nitric oxide and carbon monoxide as well as a wide variety of volatile organic compounds (see table).[1]

Typical molecules found in human breath[1]

Compound	Concentration range	Physiological basis
Acetaldehyde	ppb	Ethanol metabolism
Acetone	ppm	Decarboxylation of acetoacetate
Ammonia	ppb	Protein metabolism/bacterial metabolism
Carbon dioxide	%	Respiration
Carbon monoxide	ppm	Heme catabolism catalyzed by heme oxygenases
Carbonyl sulfide	ppb	Gut bacterial oxidation of reduced sulfur species
Ethane	ppb	Lipid peroxidation
Ethanol	ppb	Gut bacterial metabolism of sugars
Ethylene	ppb	Lipid peroxidation
Hydrogen	ppm	Carbohydrate metabolism
Hydrogen sulfide	ppb	Anaerobic bacterial metabolism of thiol proteins
Isoprene	ppb	Cholesterol biosynthesis
Methane	ppm	Gut bacterial metabolism of carbohydrates
Methanethiol	ppb	Methionine metabolism
Methylamine	ppb	Protein metabolism
Nitric oxide	ppb	Involved in vasodilatation, or neurotransmission; production catalyzed by nitric oxide synthases
1-pentane	ppb	Lipid peroxidation
Water	%	Respiration

Biomarker identification

Current research in the area of breath analysis is heavily focused on the identification of specific biomarkers in exhaled breath that can be correlated to specific disease states in the body. In addition to the presence of breath acetone in diabetics as previously mentioned, many other breath biomarkers have been discovered, including nitric oxide for airway

inflammation, hydrogen sulfide for periodontal disease, isoprene for cardiovascular disease, and numerous others.[2]

Many of these biomarkers are present in exhaled breath at extremely low concentrations, often at the part-per-million (ppm) and part-per-billion (ppb) levels. Drawing medically relevant conclusions from breath analysis requires accurate and repeatable measurements at these very low concentrations, and often with a complex mix of background gases and interferences.

Recent advances in analytical instrumentation have allowed researchers and clinicians to measure trace concentrations of these biomarkers, which have proven to be critical in the detection of normal and abnormal biological function.

While each of the established measurement techniques has its strengths and weaknesses, photonics-based breath analysis methods offer several unique advantages, including the ability to generate highly selective and sensitive results in real time. This real-time analysis provides rapid results and allows for the measurement of dynamic changes within a single breath. Real-time measurements can be especially critical in capturing the "end tidal breath," the air closest to the alveolar-capillary interface in the lung, which theoretically provides important information about metabolic processes in the blood.

Although most experts in breath analysis would claim that the field is still in its infancy, several companies have had commercial success. Most, if not all, of the products are focused on niche markets and use a highly customized sensor to assess a specific medical condition.

One example of a photonics-based product is the BreathTek Urea Breath Test (UBT) from the Medical Device Division of Otsuka America Pharmaceutical (Rockville, MD). The BreathTek UBT (www.breathtek.com) detects a biomarker in exhaled breath that indicates the presence of *H. pylori* bacteria in the gut, a leading cause of chronic gastritis and gastric ulcers. The system requires the patient to ingest a carbon-13 (^{13}C)-labeled substrate and then measures the ratio of ^{13}C to ^{12}C in exhaled breath using nondispersive IR spectroscopy. Although this has proven to be an effective product for the detection of *H. pylori* and a validation of IR spectroscopy as a viable approach for breath analysis, additional development is required to realize a more broadly applicable diagnostic instrument.

Aerocrine (Solna, Sweden) has also seen commercial success with its NIOX line of nitric oxide monitoring devices. Nitric oxide (NO) production in vascular tissue has been shown to be intimately linked with inflammation. More specifically, the presence of excessive levels of cytokines, which are linked to the body's natural inflammatory response, stimulates the production of NO in tissue. The founders of Aerocrine contributed to research in the late 1990s that showed the levels of NO in exhaled breath could be used as a sensitive biomarker for inflammation in the airways.[3] The company was founded in 1997 and now has a line of medical devices to accurately measure exhaled NO levels in the treatment of asthma and other allergic airway inflammation conditions.

The original Aerocrine devices were based on chemiluminescence but the newer devices use electrochemical sensors that have been proven to provide comparable results in a more compact, portable, and lower-cost package (see Fig. 1).[4] Although these instruments have proven to be highly effective, the Aerocrine products are another example of the confinement of current breath analysis instruments to highly specialized niche markets.

FIGURE 1. The Aerocrine NIOX MINO is a breath analysis device that has achieved the size and weight requirements for portable, handheld use. It allows for point-of-care nitric oxide (NO) measurements in the management of asthma. (*Courtesy of Aerocrine*)

Mid-IR spectroscopy, colorimetric sensors, and optical frequency combs

One active area of breath analysis research is in the utilization of quantum cascade lasers (QCLs) as light sources for laser-based spectroscopy in the mid-infrared (mid-IR) "fingerprint" region. Broadly tunable external-cavity configurations can provide access to a wide range of spectral bands where many of the breath biomarkers have unique spectral responses. Boris Mizaikoff and his team at the University of Ulm in Germany recently published their research on the successful coupling of an external-cavity QCL with a miniaturized mid-IR hollow waveguide gas cell to quantitatively determine the $^{12}CO_2/^{13}CO_2$ ratio within the exhaled breath of mice.[5]

Earlier research has shown that the isotope ratio of carbon-dioxide (CO_2) isotopologues in exhaled breath can be used as an indication of glucose metabolism dysfunction. This condition is closely correlated with septic shock, which is the leading cause of mortality in intensive care units in the United States. Research such as this is driving the adoption of optical approaches to breath analysis, which could allow for real-time online monitoring of patients in a clinical setting.

Another optical approach to breath analysis that has seen recent progress is the colorimetric sensor array. These devices use an array of chemically responsive dyes that change color based on their chemical environment (see Fig. 2). After exhaled breath is drawn across the device, an optical image is collected and analyzed to determine the complex mixture of analytes that produced the composite color response. Peter Mazzone et al. recently published the results of their study using a colorimetric sensor array to identify exhaled breath biomarkers of lung cancer.[6] The paper also reports recent advances in colorimetric sensor arrays, including robotic printing of reactive pigments to increase the surface area for reactions to occur as well as enhanced imaging technology to analyze the color responses.

FIGURE 2. A colorimetric sensor uses chemically responsive dyes to detect volatile organic compounds in exhaled breath. Image analysis of the resulting array of color determines the mixture of analytes that was present in the sample. *(Courtesy of K. Suslick, University of Illinois–Urbana-Champaign)*

In 2008, Michael J. Thorpe et al. reported the results of a more exotic optical approach that incorporated an optical frequency comb and a dispersive imaging scheme to detect trace levels of breath biomarkers in the 1.5–1.7 μm regime.[7] The instrument consisted of a gas handling subsystem, a high-finesse optical cavity, a modelocked erbium (Er^{+3}) fiber laser, dispersive imaging elements, and an indium gallium arsenide (InGaAs) camera (see Fig. 3). An innovative image-processing algorithm, involving the differential comparison of a pair of reference and absorption images, allowed the researchers to measure carbon monoxide (CO) and ammonia (NH_3) concentrations at the low ppm level as well as stable isotope ratios of carbon dioxide (CO_2). Improvements in spectral coverage and power output for frequency comb technology, coupled with high-performance imaging sensors, will allow systems such as these to approach the size and ease-of-use requirements for a clinical device.

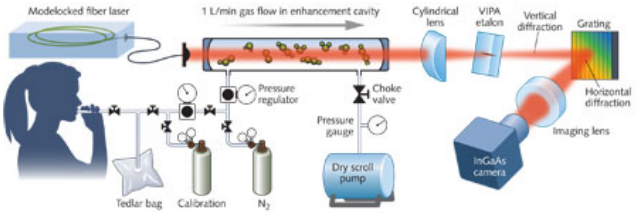

FIGURE 3. The optical approach used by Thorpe et al.[7] includes a frequency comb source coupled with a dispersive imaging scheme to detect trace levels of breath biomarkers in the 1.5–1.7 μm range. *(Courtesy of Ye Labs/JILA/NIST and University of Boulder)*

Diminishing barriers

Advances in sensor and instrumentation technology have helped to overcome some of the barriers that prevent broad adoption of breath analysis in clinical settings. Miniaturized, low-cost, portable instruments allow for the collection of breath data from large numbers of human subjects to support large, multi-institution clinical trials.[8] In addition, emerging optical approaches allow for real-time analysis of breath samples, which reduces data-processing time and enables detailed analysis of the dynamics of a single breath.

Future progress will require standardization of breath collection and analysis methods, interaction with regulatory agencies, incorporation of personalized medicine approaches, and a continued multidisciplinary focus on sensor technology development and clinical trial validation.

Cristina Davis, PhD, a professor in the department of Mechanical and Aerospace Engineering at the University of California-Davis and host of the 2012 International Breath Analysis Meeting, emphasizes the importance of collaboration between industry and academia to make meaningful progress in the area of breath analysis. "Ultimately, larger-scale breath biomarker studies will need to be performed, and I envision that this will likely need to be at the public/private interface," says Davis. "I think academic research centers will need to form a national or international consortium with industry partners to conduct the large scale clinical trials that will be needed before breath testing routinely moves into clinical practice."

Dr. Raed Dweik, a leading researcher in breath analysis and the director of the Pulmonary Vascular Program at Cleveland Clinic (Cleveland, OH), states his opinion simply with a spin on a familiar quote: "Life is not measured by the number of breaths we take, but by the ones we analyze."

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