There have been many systems of expired gas analysis indirect calorimetry developed over the years since the pioneering work done during the late 19th century. However, I would rather focus on the systems currently in use today, which have a developmental history dating back to the 1970’s. Many features of today’s systems are not that dissimilar from the procedures developed and validated since 1970. What is different is that many components have been improved with more recent advances in instrumentation.

The systems used today can be divided into three different categories.

1. **time averaged** mixing chamber systems.
2. **breath-by-breath** end tidal sampling systems, and
3. **breath-by-breath** mixing chamber systems

When I was at the University of New Mexico (UNM), we have two different types of systems; one commercial and one custom developed, but both were of the breath-by-breath mixing chamber type. However, my innovations with hardware and software in the custom system have received patent protection for their unique contributions to expired gas analysis indirect calorimetry. I will share some of these developments with you in this Topic. I have since developed another custom system at Charles Sturt University (CSU) in Australia, though it is identical to the system I built at UNM.

**Time Averaged Mixing Chamber System**

This has been the work horse system for the last 30 years because it is simple to custom develop and use. As with all electronic data acquisition systems, software was originally the main stumbling block for most researchers. This need was originally met on a commercial basis by Rick Reyfield (Reyfield Technologies, Vermont), who developed software written in computer **BASIC code** that was edited to suit specific end user needs and equipment. I used a system operated by Rick’s software during the late 1990’s (Figure 1). I also used similar systems in my Ph.D. research with David Costill at Ball State University during the late 1980’s, and my initial Masters degree study at the University of Western Australia in 1985. Today, systems are very different, but more will be presented on this later!
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Figure 2 presents the schematics of a typical mixing chamber system configuration. Ventilation measurement was typically completed using a flow meter placed on the inspired side, integrated to a potentiometer as shown in Figure 1. However, any gas flow measurement device accompanied by analog signal output of tidal volume could have been integrated to such a system. While being a heavily relied on approach to indirect calorimetry, this set-up had some frustrating flaws. First of all, there was large dead space on the expired side, with air being directed to a 3 L or larger mixing chamber used to mix expired air to get average signals of gas fractions. Thus, the dead space volume was 3 L in addition to the volume of the expired side low resistance tubing, totaling in excess of 5 L. This volume caused considerable delay between the ventilation signal and the expired gas fraction signals that was larger than typically accounted for. For example, this delay was not just a time delay equal to the sum of times between inspiration and expiration and the response time of the gas analyzers. Added to this was also the delay in having the large expired dead space air become representative of actual physiological conditions of the time of the inspired breath. As a result, data responses to changing exercise conditions sometimes took between 1 to 2 min to be evident in computations and data display.

Given these time delays, the longer the time or breathe averaging with these systems the better. I strongly believe that such limitations of this system reinforced the need to
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use the 30 s to 1 min average in data processing for all the wrong reasons (poor system design, large time and physiological delays, and software constraints).

Finally, another annoying error when using this system with a time average was how data was handled when a breath occurred across two time intervals, or just at the end or the beginning of an interval. Sometimes breaths were lost if they occurred during the computations at the end of a time interval. When a breath was aligned just prior to the end of an interval, this increased the ventilation for that interval more than prior intervals, and raised gas volumes. Conversely, if a breath occurred immediately after the start of a time interval, this lowered the previous interval ventilation, while raising the next interval ventilation. The result was that this time average approach added tremendous variability to the data, with variability increasing the smaller the time average. As you might appreciate, this was yet another reason to elongate the time averaging interval, with 1 min data looking better than any smaller time average data.

All these limitations need to be considered when interpreting data acquired from this system configuration, which unfortunately is the bulk of exercise physiology research of VO$_2$ pre-1990.

**Breath-by-Breath End Tidal Sampling System**

With the advent of the fast responding electronic gas analyzers, as well as application of the gas mass spectrometer to indirect calorimetry, there was now a rapid method for accurately quantifying expired gas fractions. Pulmonologists had originally used this equipment for profiling expired gas fractions to measure end-tidal gas fractions, which were more representative of alveolar gas fractions (Figure 3). It was a logical extension to apply this method of expired gas fraction measurement, every breath, during exercise, and hence develop the method of breath-by-breath expired gas analysis indirect calorimetry. Such systems are shown in Figure 4 for the SensorMedics Corporation and Medical Graphics Corporation.

Original validation of this method was published in the peer reviewed scientific literature in the 1980’s. Main issues that had to be dealt with for this method were as follows;

1. integration of the entire expired gas fraction curves to get the true average expired gas fractions,
2. correct timing of expired volume to gas fraction measurements using a delay factor. Here the delay factor comprised the expired air sample time to the analyzer

![Figure 3. Changes in expired and inspired air during a breathing cycle.](image)
plus the response time of the analyzer. This time alignment had to be perfect to allow valid expired signal integration over the expired breath.

3. accurate expired or inspired air flow to allow computation of accurate tidal volumes.

4. accurate and fast responding analyzers able to accurately profile an expired breath over ~300 ms during intense exercise.

I once used a commercial system based on this method, and although it worked fine after every service, or newly inserted oxygen sensor, performance always dramatically deteriorated after these events. Data became more and more questionable for high exercise intensities, such as close to VO\textsubscript{2}max, and eventually we dismantled the system, threw all the components away and kept the high quality metal cart to use with one of my custom systems!

The lesson I learned from this experience was that this approach at breath-by-breath indirect calorimetry, when using electronic fast responding gas analyzers, was not valid during more intense exercise. My understanding has been that electronic analyzers just do not respond fast enough, or perform reliably enough over time, for validly profiling the entire inspired and expired breaths when breath intervals are short, such as during intense exercise.

Richard Hughson from Canada developed a similar system more than a decade ago using a gas mass spectrometer instead of electronic gas analyzers. This makes more sense, but the intricate operation and service maintenance needs of a gas mass spectrometer makes it an expensive investment for the typical education institution teaching and research laboratory.

**Breath-by-Breath Mixing Chamber Systems**

Given the limitations of the prior method, it is no surprise that another option for breath-by-breath expired gas analysis has been devised. As you may have detected earlier, my thoughts are that the original mixing chamber time averaged system was suitable for breath by breath analyses, but was limited pre 1990 by electronic gas analyzers that simply did not have adequate response times. Today’s typical commercial mixing chamber breath-by-breath system operates essentially the same as presented in Figure 1, but with more sophisticated gas analyzers (Figure 5).
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The main issue that I do not like with this commercial system is the enormous expired dead space that has been retained. Even though the time delay may be accounted for, this is not a time delay that includes all of the necessary components (time from ventilation signal to expired breath, plus time to get to the mixing chamber, plus time for mixing, plus time for sampling to analyzers, plus response time of analyzers). The large expired dead space prevents factoring the time of expired air mixing in the dead space and mixing chamber to reflect the conditions of the expired breath. In short, it is impossible for a breath-by-breath system to be accurate in its tracking of the physiological response when there is such a large expired dead space (tubing plus mixing chamber).

Figure 5. Example of a commercial breath-by-breath mixing chamber system, where the analyzers and gas flow units are housed together in a single module. System software and hardware are controlled by a standard PC.

I have developed my system, based on improvements to all the inadequacies of the prior systems (Figure 6). To this end, I removed all added expired tubing prior to the mixing chamber by connecting the mixing chamber to the expired port of the mouthpiece. I also decreased the volume of the mixing chamber. I also developed a compliant and more elastic mixing chamber so that the quality of mixing was more similar from small tidal volumes through larger tidal volumes. I published research based on the

Figure 6. Schematics of my custom developed breath-by-breath, minimal dead space, compliant and elastic mixing chamber system.
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validation of this system in 2011.

I think it is clear from this explanation that my interpretation of the science and all published evidence is for a custom developed system. This is especially true if you can develop or attain valid software to run the system.

Glossary Words

time averaged refers to data acquired and averaged over a specific time interval.

breath-by-breath refers to data acquired and computed for each breath.

mixing chamber is a chamber, typically about 3 L in volume, that mixes expired air prior to sampling and measurement in gas analyzers for subsequent calculations of indirect calorimetry.

BASIC code is an early computer programming language that was used in numerous commercial and custom-designed software programs applied to indirect calorimetry.

flow meter is a device that measures the volume of air that flows through a bellows structure within an enclosed compartment.

potentiometer is a circular electronic device the emits voltage signals in proportion to the degrees of rotation of a central axis arm, with a typical range from 0-300º.

dead space is the volume of any additional structures that are involved in the sampling or measurement of air during indirect calorimetry.

gas mass spectrometer is an instrument that measures the content of specific gases in sampled air based on differences in the masses of the different gases.