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Towards an autonomous system with exhaled breath separation for cleaner condensed air samples

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Abstract The acquisition of condensed samples of exhaled air is a well-known noninvasive method for analyzing the healthiness of the lungs. Unlike other invasive methods as induced sputum or bronchoscopy, the condensate collection is faster and non-aggressive. During breathing, it is interesting to fractionate the sample in two differentiated portions as the first exhaled portion comes from a section of the respiratory way known as Dead Space, while the lasting exhaled portion comes from a section known as Alveolar Space. The liquid collected from the Dead Space contains a low-to-none density of biomarkers, which are mainly contained in the Alveolar Space's breathed air. Novel procedures have shown that separating the samples results in a more precise analysis of the state of the patient, improving the collected data for enhancing the current description of associated diseases. Here we describe a novel device and the associated theoretical bases for detecting and separating the exhaled air based on the source area. The implemented system integrates a closed loop for pressure control which operates on a three-ways balloon valve, based on the instantaneous exhaled amount of carbon dioxide following a proven methodology that dynamically fits the expected measured range a patient, providing a reliable cut-off among air spaces. The device, available in an open repository, besides being far less expensive than commercial devices provides a simpler and shorter method for acquiring samples.

Introduction

A very common procedure for performing pulmonary examinations through analyzing lung biomarkers is bronchoscopy, a method which can lead to damage in the breathing ways during the examination caused by either the operator of the bronchoscope or the anesthetist, necessary for executing the exam. A recent review analysis from ***DeBoer et al.*** (***2019***) shows more than 10% of procedures with complications. Compared to simpler and more recent methods, performing a bronchoscopy is highly invasive, slow and implicates elevated monetary costs.

Among recent methods, the Exhaled Breath Condensate (EBC) is a promising procedure, which disrupt the previous ways to access to the breathing areas as it only requires to have the patient to breath tidally into a closed or partially closed device. It is fully noninvasive and, as it allows to collect and post-process lung biomarkers, it can detect both respiratory and systemic diseases. Current devices implementing this technique produce samples with a low density of biomarkers, as they

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store in a single collecting cap all the condensed air, despite its origin (see a list of commercially available devices in ***Konstantinidi et al.*** (***2015***)). The collected samples, then, mainly derives from conducting airways, being highly diluted lacking the presence of biomarkers. This is a known problem of EBC and is barely assessed by requesting patients to perform slow breathing cycles, having into account that the dilution can remarkably affect the sample composition ***Horváth et al.*** (***2017***).

Here, we introduce a novel equipment which is able to separate the exhaled breath into two isolated samples: one collected sample contains condensed air from Dead Space areas (or conducting ways), depositing into another cap condensed air mostly from Alveolar Space areas. Then, this second cap contains the sample collected which is actually used for posterior biomarker's analysis, presenting a higher density and, therefor, efficacy in the collecting procedure and medical evaluation.

All codes developed for functioning and performing post-processing analysis, including examples of registered data, are available in an open repository[1].

Retrieving biomarkers from breathing areas

Condensed samples

The EBC procedure allows to retrieve biomarkers in a simple, noninvasive fashion. Standard equipment captures exhaled air in an inner camera which has to be cold enough to provoke the condensation of the contained gas. Samples are then stored as liquid or frozen material for immediate or later analysis (***Horváth et al., 2017***).

The procedure for sampling collection only requires to have the patient to breath tidally using a nose clip. While it is common to request subjects to breath over a defined period of time (e.g., 15 min.), as the collected volume depends directly on this time (***Liu and Thomas, 2007***) the absolute time per patient can variate and be defined online by the medical examiner in order to retrieve a volume sufficient enough for following examinations.

Fractionated samples

Recent proposals have shown that the efficacy in terms of density of biomarkers, measured as the number of detected biomarkers, can be enhanced in a EBC procedure by separating the exhaled air based on the proximity of its origin. The exhaled air is separated into either proximal – also known as Dead Space (e.g., trachea) – or distal airways – also known as Alveolar Space – (***Corradi et al., 2008***; ***Hoffmeyer et al., 2009***). This origin may have an important effect in the composition of the collected sample. Air from proximal airways contributes to a major dilution of the sample by injecting condensed water while it can also increase the influence of ambient air into the sample (***Reinhold and Knobloch, 2010***).

Developed equipment considering the separation of exhaled air is commercially unavailable (see e.g., ***Goldoni et al.*** (***2013***)). Its functional capabilities allow them to act as a proof of concept, but lacks of a comprehensive description obstructing its reproducibility, and therefor its usability.

Tackling this problem, here we introduce the CK Flow Divider system, a novel equipment capable of performing an automatic separation of the exhaled air into two different collecting units: one for air from Dead Space and another one for air from Alveolar Space. Using an online measure of the exhaled CO_2 based on the classical Fowler's Model for classifying the origin of the air (***Fowler, 1948***) and commercially available equipment (see following section), the CK Flow Divider system constitutes a reproducible functional device for collecting separated condensed samples.

The CK Flow Divider system: Functional Separation of Breathing Samples

The following section contains a full description of the CK Flow Divider system by introducing its composing parts and giving an architectural view. Each component, as described below, has

[1] Relevant codes and registered data presented here are publicly available at https://github.com/Sobreviviente/Fractionated_exhaled_breath.

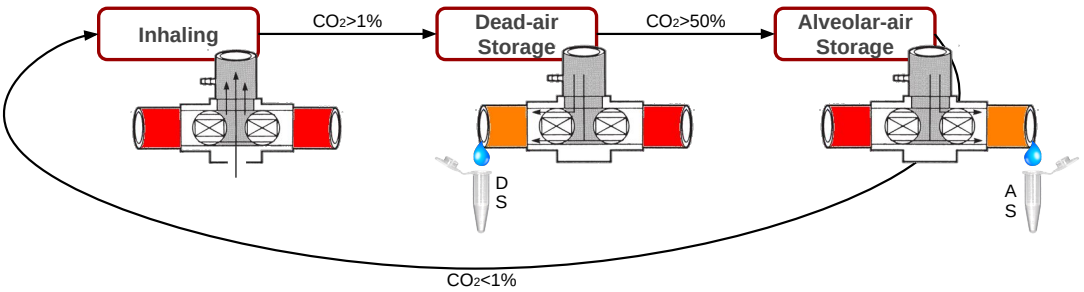

Figure 1. Sampling process for acquiring separated condensed samples. The process is separated into three stages: *Inhaling*, where air flows towards the patient isolated by a One-Way valve; *Dead-air Storage*, where the air flows to a first collecting unit (DS) in the Condenser; and the *Alveolar-air Storage*, stage where the air flow is sent to a separated, second collective unit (AS). The transition between stages depends on an online filtered estimate of the expelled CO_2.

specific responsibilities over the complete process of condensing and collecting exhaled air, as path (hydraulic) separation, control, actuation and condensation.

The CK Flow Divider is divided into four physically separable units:

1. *Flow Carrier Unit* or FCU: a set of hydraulic equipment that form the two ways for breath separation, including a T-Shape Inflatable Balloon-Type valve for flow switching.
2. *Air & Data Supplier* or AIS: a processing unit in charge of keeping a sustained air pressure for valve control, acquiring data, and computing control variables.
3. *Air Switcher* or AS: an actuator unit controlled by the AIS.
4. *Condenser*: which receives the air flow from the FCU and condense it for sampling.

The process of sampling acquisition is by itself divided into three temporally separated stages: *inhaling*, *dead-air storage*, and *alveolar-air storage*. Figure 1 shows the activation loop and control events that constitute the transition between stages. The first stage lacks sample storage as the air flow goes into the patient. The last two stages considers sample acquisition and are differentiated by which output of the FCU is open, making the air flow to reach a different collecting unit in the Condenser.

Flow Carrier Unit

The FCU integrates a mouthpiece for placing the lips, a saliva collector, a CO_2 sensor, a Three-Way T-Shape Inflatable Balloon-Type valve for separating the air flow and a One-Way respiratory valve for inhaling without contaminating the samples. The separated parts and the assembled unit are shown in figure 2 A and E, respectively. Unlike other devices, the mouthpiece can be decontaminated

Air & Data supplier

This unit contains the micro-controller in charge of monitoring and controlling the breathing and storage cycle (see figure 2 D). It has to (1) determine the stage of the breathing cycle based on online CO_2 measures, and (2) provide the air pressure for inflating the balloon valve during stage changes as shown in figure 1.

Pressure control loop for inflating the balloon valve

The Air & Data supplier incorporates a closed air circuit which has to keep a pressure above $6\ [psi] \approx 41.4\ [psi]$, nominal pressure for inflating the balloon-type valve. The pressure of the circuit is monitored using a MP3V5050DP sensor. The implemented control regulates the pressure considering hysteresis: whenever the pressure drops below $49\ [kpa]$ the system activates two rolling pump model KPM27C in charge of introducing air. The rolling pumps are deactivated when the pressure reaches $50\ [kpa]$.

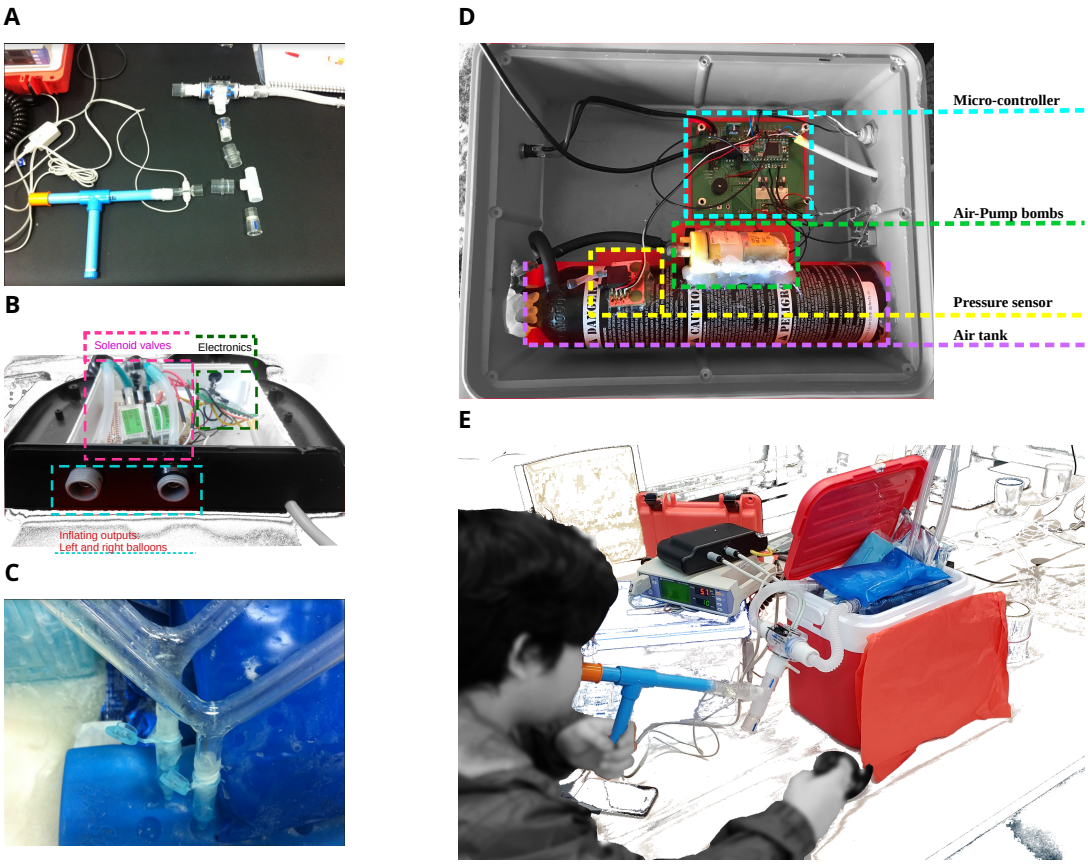

Figure 2. The CK Flow Divider system. **A** Separated parts of the Flow Carrier Unit, which compose the hidraulic air way. **B** Air & Data Supplier. **C** Air Switcher interior, which incorporates two electronically controlled valves for both air paths, enabling a controlled process of inflation and deflation of each inflatable balloon of the T-Shape valve. **D** Y-shape glass pipes with cap tubes for storing the condensed samples. **E** Complete CK Flow Divider system.

For storing the air, the closed circuit has a small tank for high pressure compressed air (shown in figure 2 D). This tank act as the air providing unit for inflating the balloons at the T-Shape valve.

CO_2-based control loop

The implemented algorithm for detecting the origin of the exhaled air is based in Fowler's model for describing Dead Space (***Fowler, 1948***). Fowler's model states that a suitable approximation for the point where the origin can be considered as Dead Space (instead of Alveolar Space) is such as when the exhaled CO_2 reaches the 50% of its maximum value.

In order to estimate the $C50$ (i.e., the point where the expelled CO_2 reaches the half of the maximum amplitude), at every breathing cycle the maximum and minimum sensed CO_2 are stored. Now, as in an online evaluation the maximum amplitude of a running breathing cycle is unknown, the CK Flow Divider uses an average of the last three stored measures as in ***Goldoni et al.*** (***2013***). Figure 4 shows the actual value and the online estimation of the $C50$. As shown, the online estimation is able to follow the actual value without introducing specific parameters for each patient. Once the $C50$ is reached, the stage passes from *Dead-air storage* to *Alveolar-air storage*, deflating and inflating the proper balloons in the T-Shape valve, redirecting the air flow from one cap tube to the other.

For monitoring the CO_2 levels, the micro-controller receives an analog signal from a Nihon Kohden OLG-2800K CO_2 monitor, which incorporates a cap-ONE CO_2 Sensor TG -920P. This signal is filtered using an exponential moving average with a smoothing factor $\alpha = 0.95$. The sampling rate

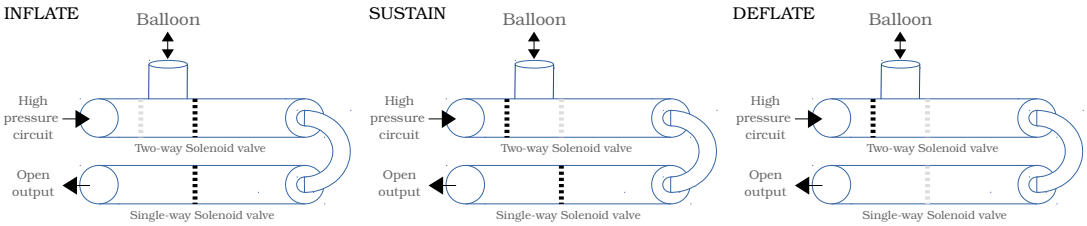

Figure 3. Functional diagram of the solenoid valves to inflate, sustain or deflate the balloons from the T-Shape valve. For controlling the inflating state of each balloon, two solenoid valves are used. The first one is a two-way solenoid valve for connecting the balloon with the high pressure circuit, inflating it. The second one allows to either sustain or deflate the balloon. This solenoids are placed inside the Air Switcher (see figure 2 B). Black and gray dashed lines show close and open connections, respectively.

for sensing the CO_2 is 680 $[Hz]$.

Air Switcher

The Air Switcher (shown in figure 2 B) is a purely actuator device fully controlled by the Air & Data supplier unit. This device contains the hardware necessary for inflating and deflating each balloon of the T-Shape valve. It receives a connection from the air tank of the Air & Data supplier, and switches between connecting a balloon either with the high pressure closed circuit for inflation, or to the environment for deflation. In order to be able to inflate and deflate each balloon separately, the Air Switcher incorporates two solenoid valves for each pathway (two valves for controlling a single balloon). The first one is a two-way solenoid valve which once activated inflates its respective balloon (see left diagram in figure 3). The second solenoid valve allows to either deflate (once open) or sustain (kept closed) the air inside the balloon (see center and right diagrams in figure 3).

Condenser

The Condenser unit (red receptacle in figure 2 E) is form by two separated sample containers (cap tubes, figure 2 C). The containers receive the air flow through a Y shaped glass pipe. Both, the container and the glass pipe, are surrounded by cold-packs producing the condensation of the air into a liquid sample. In order to keep the temperature inside a functional range, each sampling acquisition requires a set-up replacing the cold-packs, forcing to have at least two sets of them. Then, while a sampling process is being performed, the second set can be stored in a freezing unit for lowering its temperature again.

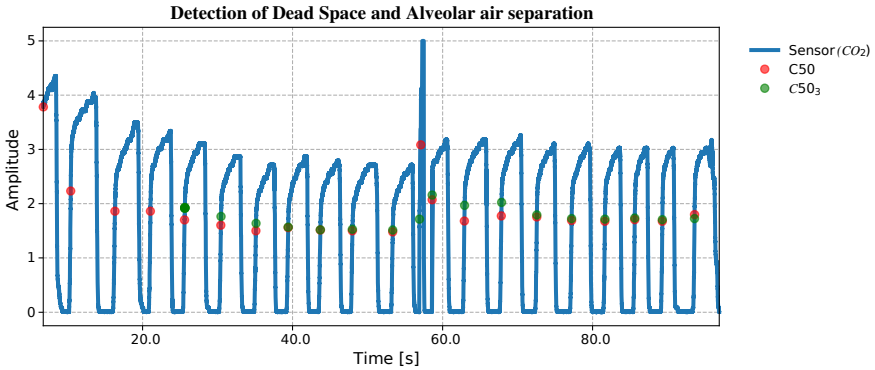

Figure 4. Online estimation of the break point between Dead-Space and Alveolar breathed air. Red dots ($C50$) show the point where the level of CO_2 reaches the half of the maximum for that breathing cycle. Green dots ($C50_3$) corresponds to the online estimation (autonomously computed as the average of last three stored half).

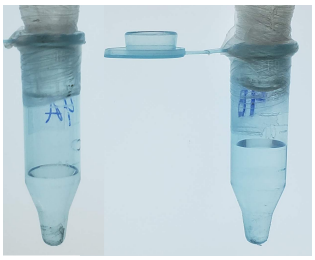

Figure 5. Condensed samples obtained through Fractionated Exhaled Breath Condensate (FEBC). The left and right containers collected Dead Space and Alveolar Space liquid, respectively. The collected sample from Alveolar Space duplicates the liquid from Dead Space.

Preliminary results

In order to ensure the precision of the detection of the $C50$ (i.e., the point where the exhaled CO_2 reaches the half of its maximum value) we have done off-line processing of registered data during a single examination. The collected data, presented in figure 4, shows 20 breathing cycles performed by a patient. Red dots in the figure shows the point for which the measured CO_2 reaches the half of the maximum for that specific breathing cycle. The figure also shows in green dots online estimated points based on the last three breathing cycles and, therefore, the point where the CK Flow Divider system changes the breathing stage and the currently inflated balloon. The presented data shows how the estimated point adjusts after a change in the maximum exhaled CO_2 (shown after a saturation of the sensor before the breathing cycle at $t = 60\ [s]$, which also do not break the estimation process). Then, all the estimated points are at least situated in the curve section associated with Alveolar air during each breathing cycle.

The CK Flow Divider has been tested by performing the full acquisition process in 8 subjects. The experiments show a continuous operation of the system with a systematic separation of the air flow. The CK Flow Divider adjusts to each patient range of expelled CO_2, ensuring the breath separation with respect to its origin: Dead Space or Alveolar Space. The condensed samples (see figure 5) show a volume difference of $50 \pm 10\%$, coherent with previous results from ***Möller et al.*** (***2010***), being Alveolar-air storage roughly twice the volume from Dead-air storage.

Discussion

Following the obtained results, in an extreme case where all Dead-air storage is condensed water from Dead Space areas, the efficacy of the condensed samples (i.e. the number of biomarkers in a certain volume) would be increase by three times. It is important to note that a posterior analysis for quantifying the amount of biomarkers has to be done to determine the absolute contribution of applying a Fractionated Exhaled Breath Condensate (FEBC).

More over, a wide testing of different materials has to be performed for characterizing the efficacy of the CK Flow Divider system for different biomarkers. Recent studies have shown how the materials for the condenser unit can alter the samples as different coating materials introduce different temperature curves (***Rosias et al., 2008***). The effect of the selection of the coating material is such as it determines which biomarkers are going to be actually present in the acquired sample. Such considerations has not been assessed in this study. The CK Flow Divider system lacks any analysis with respect to the statistical acquisition of biomarkers, which as exposed depends on the materials composing it. Once introduced the ability to store a cleaner condensed sample coming from lung spaces specifically associated with alveolar air, a fully comprehensive analysis has to be done, actualizing current knowledge about the dependency on retrieved biomakers with respect to the coating material, as it dependency could be reduced or increased. Having performed such analysis may contribute to introduce directions for standardizing separated exhaled breath condensate sampling.

Conclusions

While further characterizations of the CK Flow Divider system must be accomplished, this device presents a fully reproducible equipment for achieving Separated Exhaled Breath Condensate. The

implementation of a commercial version of this device, with the appropriate considerations for medical use, could replace a currently common invasive procedure for collecting lung biomarkers, the bronchoscopy, a method for which statistics shows an important a risk of damage ($10\ \%$ as shown by ***DeBoer et al.*** (***2019***)). Its application, through a stand-alone fully noninvasive device requiring a simple operation, can be considered for clinical environments and home health care (as currently available EBC commercial devices, see ***Konstantinidi et al.*** (***2015***)).

Some following steps for improving the hardware of the CK Flow Divider system are the integration of volume sensors, allowing to directly register the amount of exhaled volume collected into both caps separately. Others sensors for achieving a proper characterization and collection of samples are temperature sensors for monitoring the Condenser unit. Also, integrating a CO_2 sensor directly connected to the micro-controller would allow to bypass the use of a CO_2 Monitor.

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