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M. Westhoff, M. Friedrich, J. I. Baumbach

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Simultaneous measurement of inhaled air and exhaled breath by "Double-MCC/IMS".

A new method for breath analysis. Results of a feasibility study.

Westhoff M[1,2], Friedrich M[3,4], Baumbach JI[4]

[1]Department of Pneumology, Sleep and Respiratory Medicine, Hemer Lung Clinic

Theo-Funccius-Str. 1, 58675 Hemer, Germany

[2]Witten/Herdecke University, Alfred-Herrhausen-Str. 50, 58448 Witten, Germany

[3]University of Applied Sciences Münster, Hüfferstrasse 27, 48149 Münster, Germany

[4]B. Braun Melsungen AG, Branch Dortmund, Center of Competence Breath Analysis, Otto-Hahn-Str. 15, 44227 Dortmund, Germany

Correspondence

Priv.-Doz. Dr. Michael Westhoff

Department of Pneumology, Sleep and Respiratory Medicine

Hemer Lung Clinic

Theo-Funccius-Str. 1

D-56857 Hemer, Germany

Mail: michael.westhoff@lkhemer.de

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Ion mobility spectrometry, multicapillary column, exhaled breath analysis, simultaneous sampling

Abstract

The high sensitivity of methods, which are applied in breath analysis, entails a high risk of detecting analytes which do not derive from endogenous production. Consequentially, it appears useful to have knowledge about the composition of inhaled air and to include alveolar gradients into interpretation.

The current study aimed to standardise sampling procedures in breath analysis, especially with multicapillary column ion-mobility spectrometry (MCC-IMS), by applying a simultaneous registration of inhaled air and exhaled breath.

A “Double MCC-IMS” device, which for the first time allows simultaneous analysis of inhaled air and exhaled breath, was developed and tested in 18 healthy individuals. For this two BreathDiscoverys® (BDs) were coupled with each other.

Measurements of inhaled air and exhaled breath in 18 healthy individuals (mean age 46 ± 10.9 years; 9 men, 9 women) identified 35 different volatile organic compounds (VOCs) for further analysis. Not all out of these had positive alveolar gradients and could be regarded as endogenous VOCs; 16 VOCs had a positive alveolar gradient in mean, 19 VOCs a negative one. 12 VOCs were positive in more than 12 of the healthy subjects.

For the first time in our understanding a method is described, which enables simultaneous measurement of inhaled air and exhaled breath. This facilitates the calculation of alveolar gradients and selection of endogenous VOCs for exhaled breath analysis. Only a part of VOCs in exhaled breath are truly endogenous VOCs. The observation of different and varying polarities of the alveolar gradients needs further analysis.

Introduction

The high sensitivity of methods, which are applied in exhaled breath analysis, entails a high risk to detect analytes which do not derive from endogenous production. They need to be regarded as exogenous and thus confounding VOCs respectively pollutants. Therefore, exhaled breath analysis needs standardization and validation for its clinical usefulness, as postulated in European Respiratory Society (ERS) recommendations (1).

For interpretation of relevant physiological and pathological VOCs as well as circadian and day-to-day variations Wallace et al. (2) already postulated in 1996, that a greater number of collectives needs to be studied.

The relevance of variations could be shown in time series of exhaled breath by calculating alveolar gradients (3) and by times series of room air (4, 5). The inspiration of confounding, site-specific exogenous analytes may result in a transfer to the examination room and not only in their detection in exhaled breath, but also in an expiration of new analytes (4, 5). This implicates a misinterpretation of such analytes as endogenous ones. Notably, a comparison of individuals or patient groups with different diseases as well as studies at different sites bears this risk of false classification by exogenous and site-specific, but not disease-specific, analytes (5, 6). Furthermore, VOCs in room air, as well as in exhaled breath, may not only exhibit circadian fluctuations but also variations of peak intensities and alveolar gradients within longer periods of time (7).

As a consequence, for exhaled breath analysis it appears useful to have knowledge about the composition of inhaled air, mainly room air. Additionally, alveolar gradients should be included into interpretation.

First examinations about alveolar gradients by Philips et al. (8) showed that 50 % of VOCs in exhaled breath have a negative alveolar gradient. Further studies of Philips et al. (9) could detect increasing numbers of VOCs with varying proportions of negative and positive alveolar gradients. Only 27 VOCs out of more than 3.000 VOCs were consistently observed amongst the 50 healthy subjects, confirming wide inter-individual variations already in healthy individuals.

Accordingly, regarding analytes only in exhaled breath may lead to different results compared to a consideration of analytes and their peak intensities in inhaled air and exhaled breath with calculation of alveolar gradients (5). This is associated with a dramatic reduction of the number of discriminating VOCs (10).

Following the postulation of the ERS/ATS recommendations (1) for breath analysis, which outlines a framework regarding local conditions and standardization of sampling procedures we believe the best course would be to standardise sampling procedures in breath analysis with MCC-IMS. This would be done by applying a simultaneous registration of inhaled air and exhaled breath. For this we

developed a “Double MCC-IMS device”, which for the first time allows simultaneous analysis of inhaled air and exhaled breath, and we tested it in a pilot study in healthy individuals.

Methods

MCC-IMS

The study was carried out using two BioScouts®, consisting of a BreathDiscovery (BD) and a Spirometer (SpiroScout®). Therefore, the measurements of exhaled breath and inhaled air were made by ion mobility spectrometry (IMS), coupled to a multi-capillary column (MCC) (BioScout® - B&S Analytik GmbH, Dortmund, Germany). The major parameters of the MCC-IMS and of peak analysis are described elsewhere (7, 11 – 21). In the spectrometer either a 550 MBq (Breath Discovery BD 01) or a 95 MBq (Breath Discovery BD 31) ^{63}Ni ß-radiation source were applied for the ionization of the drift gas. The difference in the activity has no effect to the results because in all cases sufficient ionization was realized.

The REDMON® (B&S Analytik GmbH, Dortmund, Germany) purifies the room air to provide it as operating gas for the BD. Room air is conducted through activated carbon and a molecular sieve to dry and filter the air.

The IMS is connected to a polar multi-capillary column (MCC, type OV-5, Multichrom Ltd, Novosibirsk, Russia), which was used as a pre-separation unit.The analytes of a 10 ml sample of inhaled air respectively exhaled breath were sent through its 1,000 parallel capillaries, each with an inner diameter of 40 mm and a film thickness of 200 nm. The total diameter of the pre-separation column was 3 mm. The relevant MCC- and IMS-parameters are listed in Table 1.

Table 1: Parameters and adjustments for the Bioscouts

Parameter	Value
Sample	100 ml/min
MCC	150 ml/min
Drift	100 ml/min
Pump	0
Temperature MCC	40°C, isothermal
Polarity	Positive (+)
Humidity	Off
airflow valve	open
sample valve	sampling
Spectra count	1.500
Avarage RT	5

Parameter	Value
Avarage DT	5
Measuring program	pump
Pump flow	300 ml/min
Sampling control	Volume-controlled

Connecting a BD to a SpiroScout® (Ganshorn Medizin electronic GmbH, Niederlauer, Germany) allows a flow-triggered sample of exhaled breath. Sampling starts when a minimum volume is exhaled, which can be adjusted. Standard setting at 500 mL/min.

Breath sampling with Double-MCC-IMS (Double- SpiroScout®)

For simultaneous measurement of inhaled air and exhaled breath two BDs were used and coupled with each other. Each BD was provided with one REDMON® ((B. Braun Melsungen AG, Branch Dortmund, Germany). Because technically it was not possible to connect two BDs with one SpiroScout® or two SpiroScouts® with one BD, two independent systems were connected mechanically (Figure 1). T-pieces were custom made themselves. Therefore, the lack of marked standard mechanical possibilities was overcome, by connecting and fitting both ends with transparent adhesive tape. Normally the SpiroScout® starts breath sampling through expiratory flow signals. By rotating the second SpiroScout® 180 degrees around, it was not necessary to reverse the flow signal. As a result, the SpiroScout® displays respectively detect the flow in the different direction, which enables sampling of inhaled breath. When in- and exhaling through both fitted SpiroScouts® exhaled breath is recognized in the proximal and inhaled air in the distal one. The sampling procedure starts in that moment when both Spiroscouts® were active by the operator informing the test subject to start breathing through the sampling system. A nose clamp was used to avoid breathing through the nose. The samples of inhaled air and exhaled breath were carried to the respective BD® where further separation of the VOCs and visualization of their resulting peaks was processed.

Comparison of volume flows of inhaled air and exhaled breath

First it was tested whether both SpiroScouts® detect the flows correctly and comparably, and whether they are supplied with sufficient amounts of sample volume, the flow volumes curves of inspiration and expiration (blue lines) for each SpiroScout® were mirrored on top of each other (Fig. 2). The red line indicates the integral volume of the flow curves. The yellowish colour within the flow curve and the flow integral marks the time space within which the volume of exhaled breath and

inhaled air are above the limit (in fig. 2 a volume of 500 ml) chosen for starting the sampling procedure. All areas, resp. time spaces, marked in yellow add up to a sample duration of 10 seconds. The duration of 10 seconds is chosen to guarantee a complete air exchange within the sample loop. Additionally, the setup could be checked for possible leaks due to the new connection between the two BDs.

Numbers from one to six in Fig. 2 indicate the number of breaths during the sampling procedure. To reach the sampling time of 10 seconds four breaths were needed for inspiration, six ones for expiration. The difference between inspiration and expiration is because the duration of the expiration is longer than the one of inspiration (c.f. Δ C > Δ D). A comparison of the flow curves also shows different peak flows, with an expiratory increase to 0.75 a.u. (Δ a), and an inspiratory increase to 1 a.u. (Δ b), resulting in a higher volume per time and a shorter duration of inspiratory sampling. Because the parameters for sampling are referred to the sampling duration, but not to the volume, this results in more inspirations needed to reach the total time of 10 seconds.

Analysis of inhaled air and exhaled breath

After the BDs had finished the analysis of the samples which had been taken from inhaled air and exhaled breath, chromatograms were generated for visual interpretation as well as files that could be opened within the software VisualNow® (B&S Analytik GmbH, Dortmund, Germany). The measurement files are visualizing the three dimensions, peak positions by drift and retention time and the peak intensities.

The feasibility of the method was first proved in a single healthy person by analyzing 12 selected VOCs.

Afterwards 18 healthy persons had simultaneous analysis of inhaled air and exhaled breath by the presented Double-MCC-IMS method.

Results

Comparison of single heat maps of inhaled air and exhaled breath

The heat maps (Fig. 3) represent the spectra of drift time and retention time and the peak heights of a single individuum. The peak heights correlate with the intensity of the VOCs, which is underlined by the colour range; with white being the lowest and yellow the highest intensity. In the left figure, the analytes of inspiration are shown, in the right those of expiration. For a better comparison of product ions in inhaled and exhaled air, the RIP was cut out.

The product ions of inhaled air and exhaled breath differ in their peak intensities (Fig. 3). Peak 5 in inspiration is visually recognizable as more intense (cf. black frames). In comparison to that, P1 in expiration is more intense (cf. black frames). However, it is not always the case that one can

distinguish peak differences so clearly visually. Therefore, the indication and evaluation of the numeric peak intensity values is needed.

Comparison of inhaled air and exhaled breath in 18 healthy persons

18 healthy persons (mean age 46 ± 10.9 years; 9 men, 9 women) had measurements of inhaled air and exhaled breath for comparison. 35 different peaks could be identified and were put together to a set (Fig. 4). The horizontal rows show the 35 different peaks found in every person. The 18 upper rows correspond to the patients' inspiration, the lower 18 rows to their expiration. Peak intensities reach from white being the lowest to yellow being the highest intensity. Remarkable differences of peak intensities of exhaled breath and inhaled air are already detectable visually e.g., at peak P1. This peak is more intense peaks on inhaled air compared to exhaled breath (c.f. black frame).

Differentiation of endogenous VOCs

The visual comparison already shows a significant difference of single peak intensities between inspiration and expiration ($p < 0.001$). For more precise evaluation alveolar gradients from peak intensities of exhaled and inhaled VOCs were calculated.

The calculation showed, that not all out of 35 peaks in exhaled air had positive alveolar gradients and can be regarded as endogenous VOCs.

For example, in case of P1 in the second column of Fig. 4 the measurements of inhaled air in nearly all patients show a dark blue to reddish spot, whereas in contrast in exhaled breath only light blue spots are present. Regarding the measured intensity values, Table 2 shows the mean positive alveolar gradients of 16 peaks. 12 of these peaks were positive in more than 12 of the healthy subjects, 5 peaks in more than 15 of them.

Table 2: Mean positive alveolar gradients (green numbers) of the 16 peaks with standard deviation (SD).

Peak	**P10**	**P11**	**P13**	**P14**	**P20**	**P23**	**P24**	**P26**	**P27**	**P28**
alveolar gradient	0,0223	0,0204	0,0030	0,0293	0,0930	0,0005	0,0013	0,0011	0,0025	0,0009
SD	0,0285	0,0261	0,0025	0,0266	0,1208	0,0009	0,0087	0,0104	0,0103	0,0057
Peak	**P32**	**P35**	**P36**	**P5**	**P7**	**P9**				
alveolar gradient	0,0008	0,0011	0,0011	0,0045	0,0242	0,0100				
SD	0,0023	0,0049	0,0046	0,0150	0,0448	0,0288				

Table 3 shows the mean negative alveolar gradients of the remaining 19 peaks. 16 of these peaks were negative in 15 or more of the healthy subjects.

Table 3: Mean negative alveolar gradients (red numbers) of 19 peaks with standard deviation (SD)

Peak	**P0**	**P1**	**P12**	**P19**	**P2**	**P21**	**P22**	**P25**	**P29**	**P3**
alveolar gradient	-0,0031	-0,0076	-0,0306	-0,0076	-0,0243	-0,0007	-0,0005	-0,0042	-0,0083	-0,0032
SD	0,0031	0,0040	0,0352	0,0038	0,0273	0,0022	0,0067	0,0053	0,0058	0,0033
Peak	**P30**	**P31**	**P33**	**P34**	**P37**	**P38**	**P4**	**P6**	**P8**	
alveolar gradient	-0,0015	-0,0284	-0,0026	-0,0016	-0,0009	-0,0002	-0,0048	-0,2429	-0,0781	
SD	0,0036	0,0326	0,0022	0,0030	0,0036	0,0042	0,0078	0,0927	0,0685	

Discussion

For the first time in our understanding, by using a Double MCC-IMS, we describe a method, which enables simultaneous measurement of inhaled air and exhaled breath, thus facilitating the calculation of alveolar gradients and selecting endogenous VOCs for exhaled breath analysis.

By applying the presented method, it can be shown that only a part of VOCs in exhaled breath are truly endogenous VOCs. More than half of the VOCs chosen for analysis had negative alveolar gradients and only 16 peaks had positive alveolar gradients in a mean. However, only 5 peaks had positive alveolar gradients in most subjects, and only 2 peaks in all subjects.

This finding has an important impact on the interpretation of VOCs. This concerns their metabolism as well as their relevance for disease classification. As Philips et al. (8, 9, 22) already showed, considering only exhaled VOCs does not lead to the conclusion, that all VOCs in exhaled breath represent truly endogenous ones. The simultaneous measurement of inhaled air and exhaled breath by Double-MCC-IMS certifies this. Even most of the detectable VOCs in exhaled breath have lower intensities than in inhaled air and cannot be regarded as endogenous ones. This confirms the data of Pizzini et al. (10), that the number of discriminant VOCs, which can be used for exhaled breath analysis, is much lower than the number of VOCs detectable in exhaled breath when calculating alveolar gradients. However, in contrast to our simultaneous analysis Pizzini et al. (10) used a separated analysis of room air and exhaled breath.

The observed negative alveolar gradients, which result from higher peak intensities in inspiration than in expiration, may have different reasons. VOCs may either be attributable to originary room air (4), other external factors like clothes and perfume or to VOCs which are transferred by the patient from former locations to the examination room (5). Contaminations caused by a disinfectant at a location different to the examination room were shown (5) to result in significantly higher intensities of VOCs not only in exhaled breath but also in the examination room, compared to corresponding

baseline measurements. Such constellations often occur in hospitals where disinfectants are routinely used. This strengthens the necessity to evaluate either if inhaled air is possibly contaminated by such confounding analytes or if newly detected VOCs are a result of an endogenous metabolization or possibly an induction of an inflammatory process by such irritant analytes.

Furthermore, as was seen in indoor time series (3, 4, 23), indoor VOCs are dependent on room airing and may exhibit different behaviours. In this case, the concentration decreases, increases or changes cyclically over time

Gaida et al. (6), even after having excluded confounding cleaning agents, still found locational differences and features that influenced exhaled breath analysis in COPD-patients. Thus, by comparing persons or patient groups with different diseases as well as studies at different sites, this could implicate a risk of misclassification, if VOCs are exogenous and caused by the location, but not disease specific. This confirms the relevance to get additional information about local analytes and their influence on room air, especially inhaled air, and on exhaled breath.

These particularities not only have implications on the interpretation of exhaled breath but also highlight the necessity to overcome these confounding influences by a method-specific standardisation of the sampling procedure. For this the constant ventilation of the examination room with fresh air and especially the inspiration of synthetic air were regarded as appropriate to overcome such exogenous influences (5, 24). However, much more than a constant ventilation of the examination room with fresh air, and beyond a wanted reduction of confounding exogeneous analytes, inspiration of synthetic air may also reduce relevant endogenous analytes (4, 5). This - as well as reported variations of peak intensities in synthetic air (23) - can influence the height and polarity of the alveolar gradient (4, 5).

A further approach is the calculation of alveolar gradients by analysing room air, as was done mainly by Philips et al. (8, 9, 22, 25, 26). They found that 50 VOCs with the highest alveolar gradients mostly comprised benzene derivatives, acetone, methylated derivatives of alkanes, and isoprene (27). Up to now only a few further studies regarded alveolar gradients (3, 10, 28, 29). They also calculated them by measuring exhaled breath and separately room air. However, when room air was used as a reference, it was assumed that inhaled air not only contains the same VOCs, but also with the same concentration as room air. But as we could show in another study by using Double-MCC-IMS (30) this does not provide reliable information about inhaled air. The composition of room air and inhaled air may not be identical; but if so, the VOCs which are detected in both may have different peak intensities, thus potentially even leading to different alveolar gradients. Therefore, simultaneous measurements of inhaled air are preferable.

The interpretation of negative alveolar gradients is challenging. Negative gradients do not necessarily mean that the related VOCs are not valuable for further interpretation. The degree of their reduced

intensity, either because of metabolization or absorption, might provide additional and valuable information. In case of pentane in normal subjects Philips et al. (22) made a subdivision into "passive equilibrators" who did not appear to excrete pentane in the breath and represent the majority, into "metabolizers" who actively catabolized inhaled pentane, and into "manufacturers" who excreted more pentane than they inhaled. Furthermore the gradient was found higher in cystic fibrosis patients, especially in those with exacerbations, than in healthy controls, with an inverse proportionality to forced expiratory volume in one second (31). Pollutants, i.e. exogenous compounds, in inhaled air are partially retained in the exhaled breath and were found to follow a close, compound speficic linear relationship between the exhaled and inhaled air concentrations (32). However, there are no further conclusive data. In the future, simultaneous measurement of inhaled air and exhaled breath may offer further insights, especially concerning the metabolization and resorption of VOCs, a process which may also be disease specific.

When calculating alveolar gradients, a further question arises concerning the minimal significant intensity, which allows to regard VOCs as relevant ones for further interpretation of exhaled breath analysis. Therefore, VOC-specific cut-off values need to be defined, which exclude analytes, if their alveolar gradients fall below them. Some authors (28, 29) only included VOCs, if they had a concentration in exhaled breath that was at least 15 % higher than in room air, like a threshold. This seems arbitrary, because the significant difference and the polarity of the alveolar gradient may exhibit variations over time (3, 5, 33), even when the sampling procedure is standardised. Besides that, the forementioned studies only used room air instead of truly inhaled air for calculation of alveolar gradients.

Unfortunately, hitherto there is no consensus, if alveolar gradients (25, 26, 28, 29, 34, 35) or absolute concentrations respectively peak intensities of analytes (36 – 38) should be used in exhaled breath analysis. With reference to such complex interactions between expiration and uptake of VOCs some authors (37, 38) even doubt a simple subtraction of peak-intensities to be appropriate.

However, standardized simultaneous measurement of alveolar gradients by a method like “Double-MCC-IMS” may open a new field and give further answers to these questions.

There are some limitations of our study. We used two BDs of different production series. This may have an influence on the peak intensities. Further studies using test substances are necessary to ascertain, that measurements with both BDs result in identical peak intensities.

Furthermore, the integral volumes of both devices at the first breath differed about 10 %, with the inspiratory volume being less. This is probably due to a greater dead space between the mouthpiece and the distal BD, which measures inspiration, compared to the proximal BD and the mouthpiece. Because the volume flow process matches, variations of about 10 % might be neglected. Otherwise, we cannot exclude, that neither differences up to 10 % nor a possible rebreathing of exhaled breath

out of the dead space influence the alveolar gradient. This might explain that between some subjects the alveolar gradients of the peaks varied. A daily variation of alveolar gradients had already been described by Bunkowsky et al. (3) in time series when calculating alveolar gradients by measuring room air. In order to exclude the position of the BDs, which was chosen for in- and expiratory measurements, as an influential factor, additional measurements with a switch of both BDs may give further insights. Connecting both SpiroScouts® by an Y-piece with an inspiration and expiration valve is a further option to reduce dead space and to prevent a possible influence of a rebreathing effect on the alveolar gradient.

Furthermore, the connection of both SpiroScouts® might be a potential source of leakage.

The calculation of alveolar gradients is not yet automatized. However, this can be overcome in the next steps by developing special computerized programs.

We only tested healthy individuals. However, data about exhaled breath analysis alone and additionally calculated alveolar gradients in a comparison of healthy individuals and COPD patients are in preparation. So, it can be determined, if alveolar gradients provide a higher sensitivity and specificity than an analysis of exhaled VOCs alone.

In conclusion we present for the first time a feasibility study about simultaneous measurement of inhaled air and exhaled breath by using a “Double MCC-IMS”-device. The first data show, that an onsite calculation of alveolar gradients by this method may allow a more precise selection of truly endogenous VOCs for exhaled breath analysis.

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Ethics statement

The study was approved by the ethics committee of the University of Münster and registered in ClinicalTrials.gov (NCT00632307) and the German Registry of clinical studies (DRKS00000026). Consent to participation was provided through written consent.

Conflict of Interest Statement

MW declares to have no relationships with any industry or competing conflicts of interest. MF and JIB are members of the staff of Braun Melsungen AG, Branch Dortmund, Center of Competence Breath Analysis, Otto-Hahn-Str. 15, 44227 Dortmund

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Authors' Contributions

MW, MF, JIB contributed to the development of Double-MCC-IMS equally. MW and MF contributed to data acquisition. MW, MF and JIB contributed to search and review of literature, drafting and revising the final draft. All authors have read and approved this manuscript.

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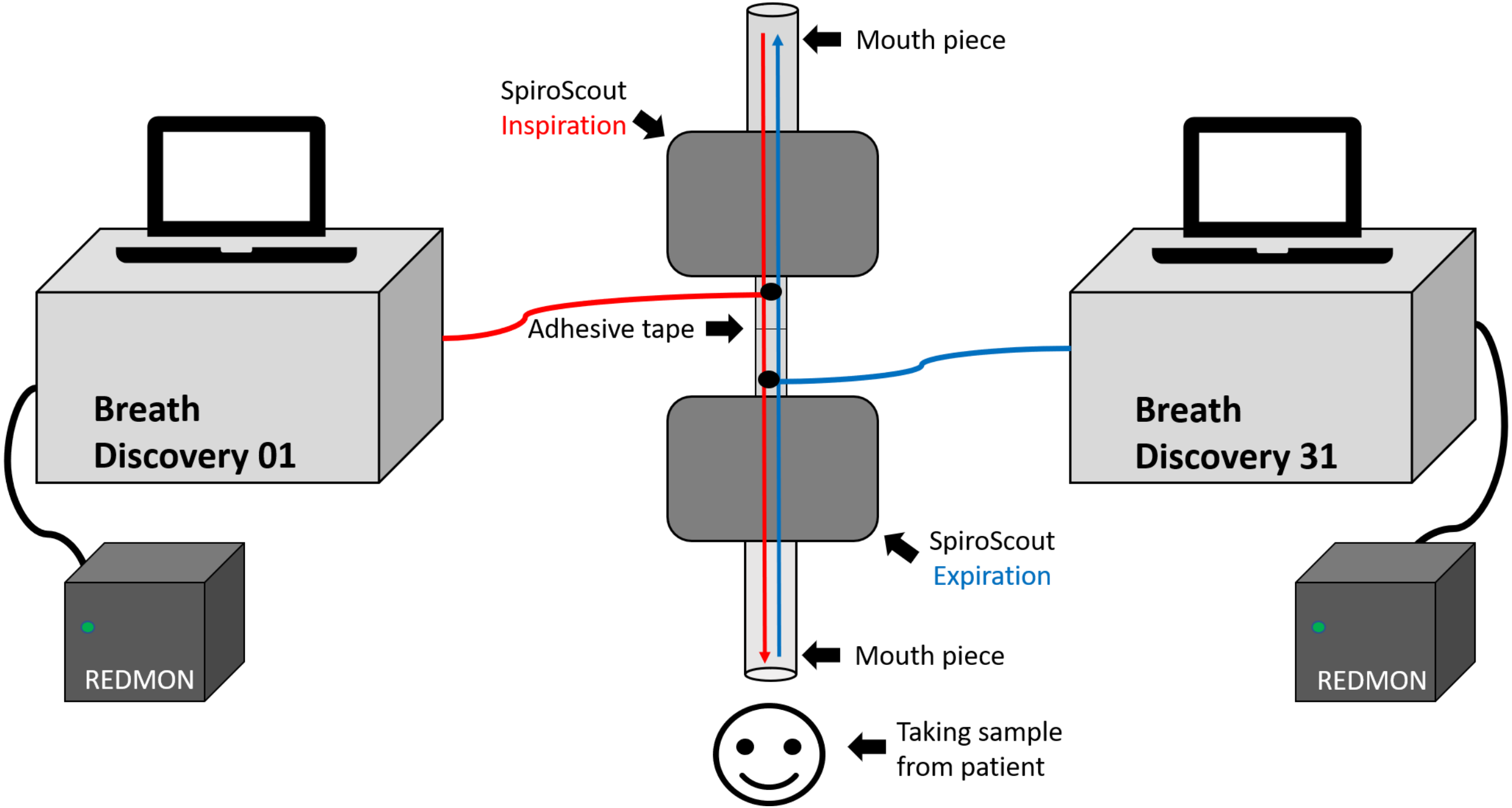
Figure legends

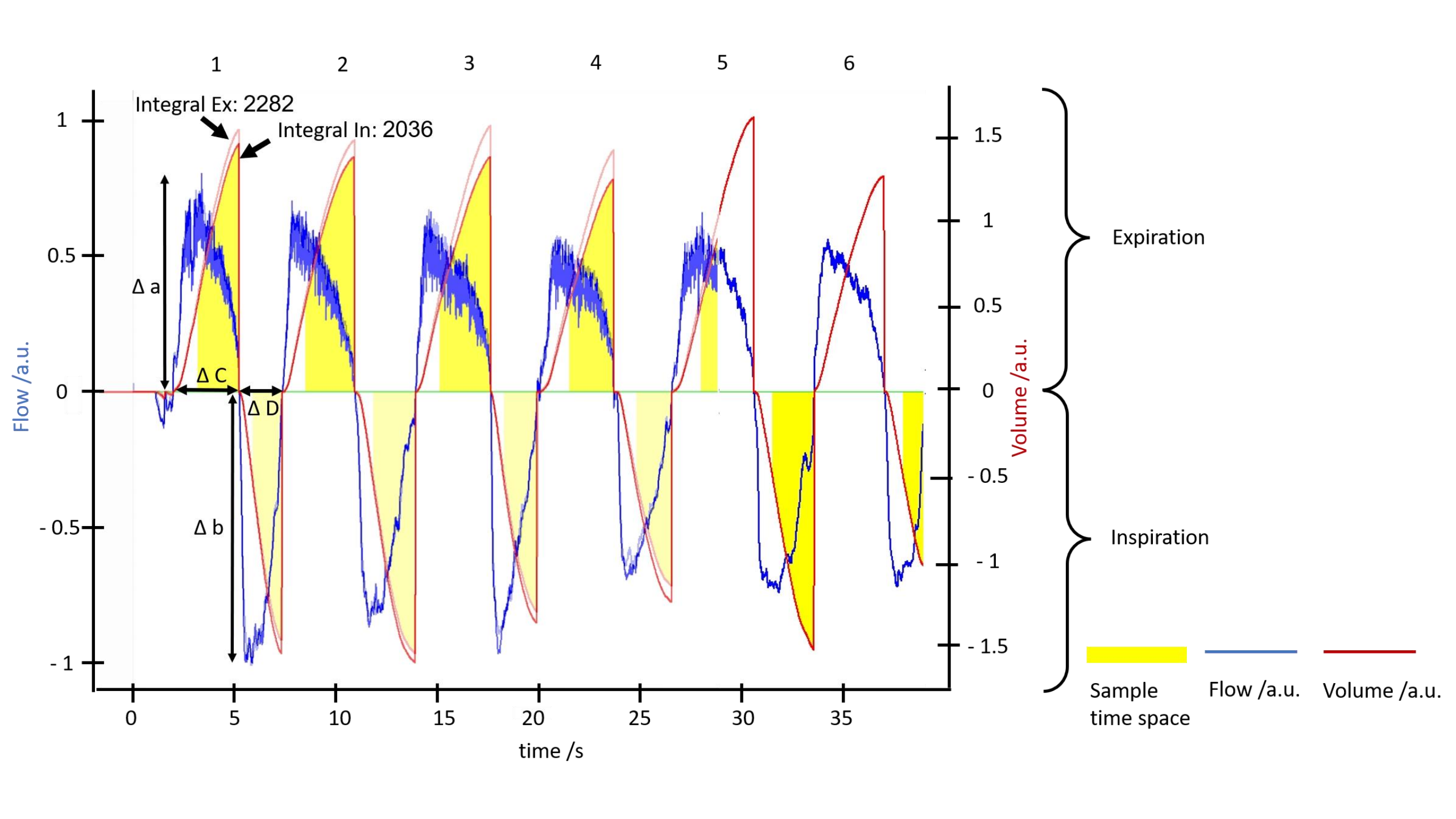
Figure 1: Schematic experimental setup consisting of two Breath Discoverys (BD), REDMONs and SpiroScouts®. The rear SpiroScout® was turned around 180 degrees and connected to the proximal one. Each SpiroScout® is linked to one BD, that is connected to a REDMON, that provides the operating gas. The red line indicates the way of the inhaled air moving towards the patient through the SpiroScouts® and reaching the BD01. The blue line shows the way of exhaled breath moving away from the patient and reaching the corresponding BD.

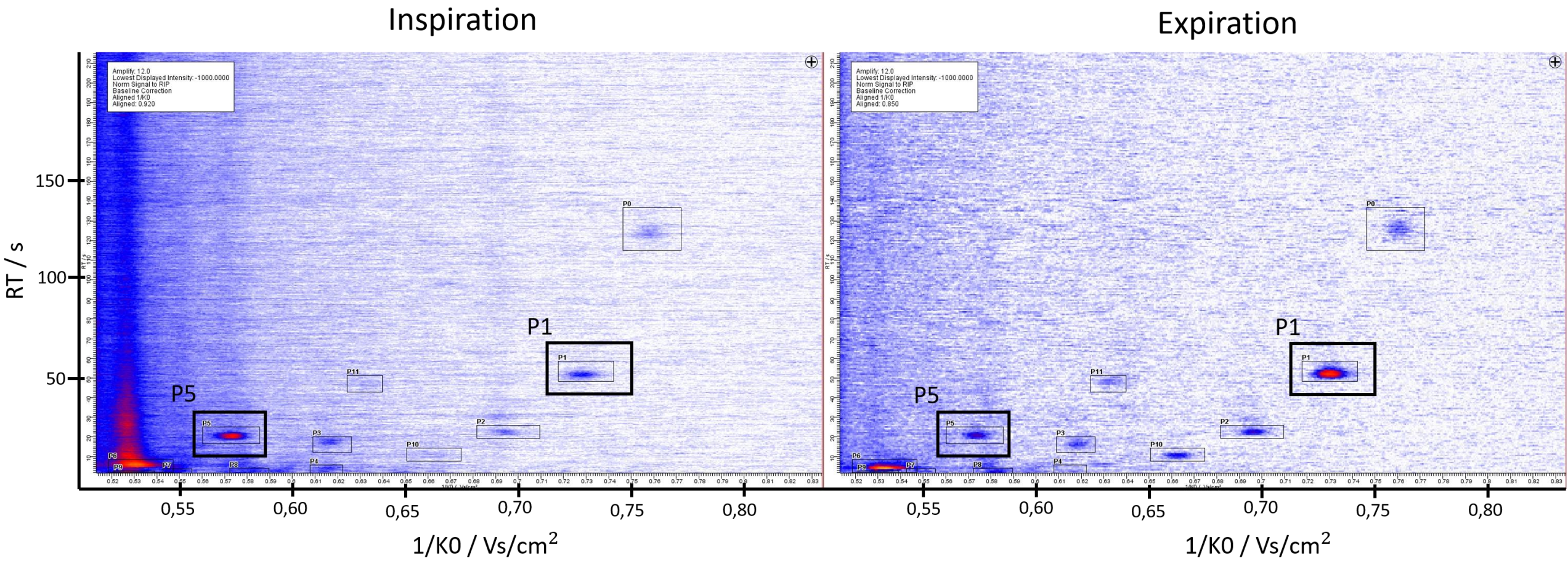
Figure 2: Comparison of volume and flows during inspiration and expiration by mirror them on top of each other. On left y-axis the flow (blue line) in a.u. (arbitrary units) is shown, while the right y-axis (red line) indicates the volume (flow rate over the time), also in a.u. The time in seconds is shown on the x-axis. The flow is proportional to l/s and the volume to l. Though, instruments are not calibrated, they are equal. Δ a and Δ b indicate the rise of the flow at expiration and inspiration, respectively. The duration of either expiration or inspiration is indicated by Δ C and Δ D. Each SpiroScout should recognize both directions of flow, even if only one is collected and analysed. The area of the integrals Ex (expiration) and In (inspiration) show that although their flows are mirrored and should be the same, they slightly differ from each other.

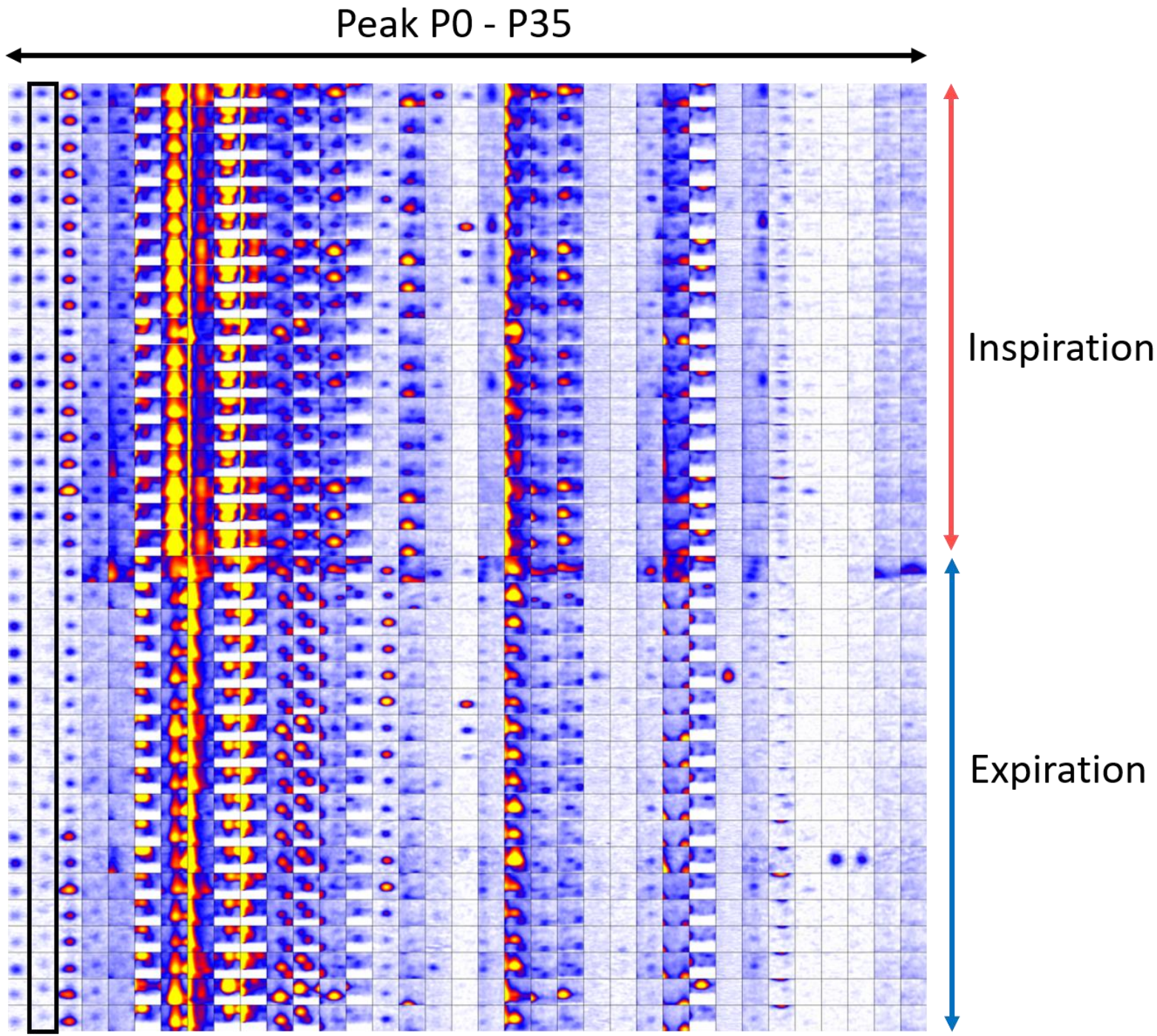
Figure 3: 3D spectrum of inhaled air (left) and exhaled breath (right). y-axis indicates the retention time (RT) in seconds (s), the x-axis the drift time in 1/K0 Vs/cm^2. The third axis the peak intensity increases from white to yellow colour. At inspiration peak P1 alone can be visually recognised as more intense compared to the expiration. Peak P5, however, can be seen more intensely during expiration (cf. black frames). For detailed peak analysis intensities have to be measured and compared.

Figure 4: Peak images of all peaks found from all 18 healthy test persons. The images are created from the peak windows from the 3D spectrum of each measurement. Peak intensities reach from white being the lowest intensity to yellow being the highest. The top18 rows result from inhaled air (rows at the height of the red arrow), the lower 18 show exhaled breath (rows at the height of the blue arrow). Each column shows one out of 35 peaks, ascending from left to right and starting at peak P0.... Black frame circles peak P1. Here the difference between the more intense peaks on inhaled air compared to exhaled breath can be made visually.


Mouth piece
SpiroScout
Inspiration
Adhesive tape
Breath
Discovery 01
Breath
Discovery 31
SpiroScout
Expiration
Mouth piece
REDMON
REDMON
Taking sample
from patient


1
2
3
4
5
6
Integral Ex: 2282
Integral In: 2036
Δ a
Δ C
Δ D
Δ b
Flow /a.u.
Volume /a.u.
1
0.5
0
- 0.5
- 1
1.5
1
0.5
0
- 0.5
- 1
- 1.5
0
5
10
15
20
25
30
35
time /s
Expiration
Inspiration
Sample time space
Flow /a.u.
Volume /a.u.


Inspiration
Expiration
Amplify: 12.0
Lowest Displayed Intensity: -1000.0000
Norm Signal to RIP
Baseline Correction
Aligned 1/K0
Aligned: 0.920
Aligned: 0.850
RT / s
150
100
50
P0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
0,55
0,60
0,65
0,70
0,75
0,80
1/K0 / Vs/cm2


Peak P0 - P35
Inspiration
Expiration