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REVIEW ARTICLE

The application of FAIMS gas analysis in Medical Diagnostics

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There is an ever increasing need to develop new tools to aid in the diagnosis and monitoing of human diseases. Such tools will ultimately reduce the cost of healthcare by identifying disease states more quickly and cheaply than current practices. One method showing promise is the analysis of gas-phase biomarkers from human breath, urine, sweat and stool that reflect bodily metabolism. Analysis of these volatiles by GC MS requires specialised infra-structure and staff, making it unsuitable for a clinical setting. Point of care sensor based technologies such as eNoses often suffer from stability and sensitivity issues. Field-Asymmetric Ion Mobility Spectrometry (FAIMS) has potential to fulfil this clinical need. In this paper we review the medical need, the technology, sampling methods and medical evidence thusfar. We conclude with reflecting on future developmental steps necessary to bring the device into medical practice.

Introduction

The last 100 years has seen a huge increase in new technologies and approaches applied to medicine. Hospitals are now filled with a plethora of analytical instruments that can aid the clinician in making an informed choice of the appropriate treatment pathway. However, many of these techniques are still focussed towards secondary care and require the use of expensive, dedicated facilities to undertake diagnosis. However, with the drive to reduce medical expenditure, we are seeing an increase in point-of-care approaches moving diagnostics from secondary to primary care and even the home.

One approach which has been mooted to aid in this change has been the use of gas phase biomarkers. It is well known that these volatile organic compounds (VOCs) are the end product of metabolic processes in the body that are modulated by a variety of diseases[1]. The best known example perhaps is the exhalation of acetone by diabetic patients during ketoacidosis[2]. Because, many of these compounds can be detected in normal human samples, (such as breath, urine, stool, blood or sweat) analysis of volatile biomarkers is non-invasive, offering a route into primary care. This makes VOCs very attractive biomarkers for monitoring, diagnosis and prognosis of disease. The research challenge of using such markers is similar to that found in many other 'omics' techniques[3]. The high dimensionality of the data means it is challenging to identify the biomarkers in the background. Furthermore many VOCs are highly unstable meaning they are easily affected by altered measuring conditions. Finally to facilitate implementation of these techniques, the analysis should not require specialised laboratory facilities or significant infra-structure (e.g. specialised gases), be easy to operate and have both a low instrument and unit test cost.

Despite it's potential there are currently no tools implemented in clinical practice working on the basis of VOCs. Much of the previous work in this field has deployed gold-standard methods for gas analysis, focussing much on GC/MS (Gas Chromatography/Mass Spectrometry) and similar techniques. However, such instruments have several limitations for many clinical settings. These techniques require extensive training and expertise and expensive equipment making these procedures costly and time-consuming. Furthermore the nature of sample introduction and data-analysis precludes it from being used as a point of care tool. The latter is desired for many medical applications, especially those focusing on infectious disease and monitoring of chronic inflammatory conditions. These reasons have sparked considerable interest in using instruments such as the "Electronic Nose", based on arrays of discrete, non-selective sensors, for disease diagnostics. At first glance, these instruments fulfil many of the requirements for a medical scenario. They are relatively cheap, use air as the carrier, are simple to use and can be reduced in size for point-of-care use. The downside of these approaches is that many of these devices unfortunately suffer from poor intra-device repeatability, limited temporal stability and poor selectivity due to the promiscuous nature of the chemical interactions between the sample and the sensors[4]. This means translation to clinical practice is severely hampered: a diagnostic algorithm will have to be built separately for each device rather than transferring data from one device to another. More recently, ion mobility techniques have shown promise. They provide high information content, physical measurement of complex chemical headspaces, but still fulfil

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many of the requirements needed as a point of care tool. One specific ion mobility method receiving considerable interest is FAIMS (Field Asymmetric Ion Mobility Spectrometry). Its principle is based on assessing the physiochemical properties of VOCs, which means compounds can be selectively identified in a complex background. Furthermore, detectors show good intra device stability allowing a plug-and-play application in clinical practice after the initial validation. In this article we review previous work using FAIMS applied to the medical sector and lessons learnt for its practical application.

Ion Mobility Spectrometry

When an ion in a vacuum is exposed to an electric field, it experiences a constant force, F=qE, and its motion will depend only upon its mass and charge. However, if it is surrounded by a buffer gas, when the electric field is applied, collisions with the atoms of the gas will rapidly cause it to stop accelerating. Its terminal velocity, v, is related to the electric field strength, E, by v=KE, where K, the ion's mobility, depends on its collision cross-section. An ion with a larger cross-section will collide more often, and hence will have a lower terminal velocity, and lower K. For a given ion, K is not a constant, but is instead a function of the electric field strength i.e. K=K(E). Ion mobility spectrometry (IMS) uses measurements of ions' mobility to identify them.

Field Asymmetric Ion Mobility Spectrometry (FAIMS)

Field asymmetric ion mobility spectrometry (FAIMS) exploits the fact that by applying an alternating electric field across the path of the ions, a filter can be set up that allows only certain ions through. Vapour from a sample is first ionized, and then passed between two parallel plates. An alternating voltage is applied across the plates, creating an alternating electric field in the space between them. The form of the voltage, shown in Figure 1a, is a short-duration, high-amplitude positive section followed by a long duration, low-amplitude negative section. The electric field is applied in a direction perpendicular to the ions' initial direction of travel through the channel, which will give an additional velocity v=KE to the ions in the direction of the field. As a result, the ions will travel along a saw-tooth trajectory in the channel, as shown in Figure 1b. The alternating voltage is designed so that the product of the voltage and the pulse duration is the same for the positive (pink in Figure 1a) and negative (blue in Figure 1a) sections. This means that if the ion's mobility, K, is the same under high- and low-field conditions, then the ion will move upwards during the positive section the same amount as it moves downwards during the negative section, giving a net vertical movement of zero, and allowing the ion to pass through the channel (along the path marked 1 in Figure 1b). However, as mentioned earlier, the mobility will in general not be constant, but will instead vary with field strength. If the mobility increases at high field strength, the ion will travel further vertically during the high-field phase than the low-field phase, and follow a path like that marked 2, while a decrease in mobility with increased field strength will result in an ion following a trajectory like that marked 3. An ion's path, then, is determined by its differential mobility ($K_{high} - K_{low}$); the greater the difference between the ion's high- and low-field mobilities, the more quickly it will hit the sides of the channel and be lost. Some examples of the ways in which differential mobility varies with field strength are shown in Figure 1c.

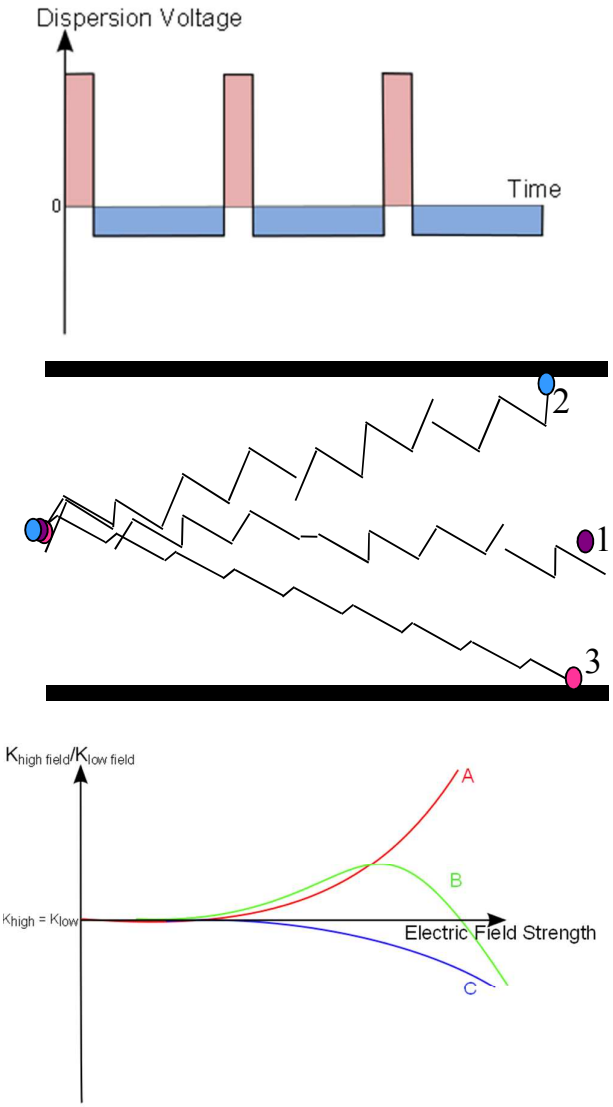

Figure 1: (a) Alternating Square Wave, (b) Ion trajectories in FAIMS and (c) Variation of mobility with increasing field strength.

Selecting Ions

In order to control which ions pass through the system, an additional DC voltage can be applied between the plates. Often referred to as the compensation voltage (CV), its value can be tuned so as to exactly compensate for the net vertical

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drift of a particular ion. By scanning through values of the CV, and recording the ion current emerging from the channel at each, we can detect each of the ion types present. The magnitude of the AC signal may also be altered, meaning the strength of the electric field (also known as the dispersion field, DF) in the high- and low-field sections can change (although the ratio remains constant). Because the mobility is a complicated function of field strength, this will result in a change in the differential mobility for each ion. This is important because a) as can be seen from Figure 1c, at low field values, the differential mobility for all ions is the same (≈0) and b) even at high field values, two particular ions may happen to have the same differential mobility at a particular DF value (as can be seen from the fact that the lines marked A and B cross in Figure 1c). By varying the DF, we can generally find a DF value at which compounds can generally be separated, as shown in Figure 2. By scanning through the range of both DF and CV values, and recording the ion current at each, we can generate a data-rich chemical fingerprint, containing information about all of the compounds present in a sample. An example of this can be seen in figure 4 below

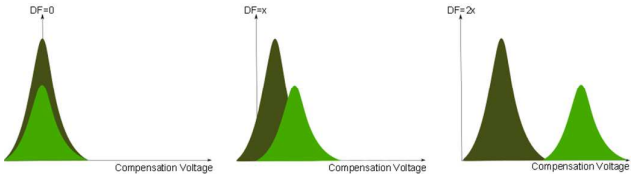

Figure 2: Varying the dispersion field alters the compensation voltage necessary for different compounds to pass through the system.

Experimental application of FAIMS

Two key components with any analytical approach are the sampling and how the data are analysed. In this section we consider these components.

Sampling human volatiles

Current medical studies with FAIMS have focussed predominantly on urine, breath and stool samples. Of these urine has been the main biological medium of choice – predominantly due to its high patient acceptability, ease of collection and better chemical stability. As FAIMS has yet to make it into clinical practice, most samples are collected and then frozen for later batch sampling. Stool, though less stable, is composed of far more chemical components, and a similar collection and testing regime can be applied to produce reliable results. An example collection and testing regime for urine and stool is provided below:

- Samples require putting on dry-ice/fridge immediately on collection;
- Frozen at -80 ^{o}C within 2 hours;
- Defrosted to fridge temperature and aliquoted at that temperature (to reduce chemical loss);
- Use a sample volume between 4 and 10 ml;
- Tested immediately after aliquoting to reduce chemical loss through increased temperature;
- Analysis of samples at body temperature (37-40 ^{o}C), first allowing the sample to reach temperature over a 10 minute period.

Ideally, samples should be tested using a standardized sampling system (for example Owlstone ATLAS system). Such a system aids in controlling the temperature of the sample, allows for the accurate control of the flow rate into the instrument and can have heated transfer lines to minimise chemical adhesion to transfer tubes. Another consideration with stool and urine samples is the high water content, which can mask more subtle chemical components. Diluting the air from the sample with clean, dry air (for example to a ratio of 1:3 – 1:4) reduces its effect resulting in increased sensitivity.

Breath sampling brings its own additional challenges. The direct introduction of a breath sample into a FAIMS instrument is challenging, due to the difficulty in controlling the sample flow from the subject. Though there is not a significant body of work using FAIMS and breath samples there are two common approaches to overcoming this problem. The first is to use a breath capture system whereby end-tidal breath is captured in an inert collection bag (such as Tedlar,shown in Figure 3). This sample can then be introduced into the instrument, thereby separating the collection from the testing. It is worth noting that previous studies have attempted to test the samples within a few hours of collection, probably due to breakdown of the chemical components in the bag. A second approach consists of storing breath samples onto chemical sorbent tubes such as Tenax bypassing the need for immediate analysis until at least 14 days past sample collection[4]. An international consortium of researchers is currently developing an open access breath collection device aiming to provide a flexible and reproducible method for breath collection www.breathe-free.org.

Because biological symptoms are inherently unstable, so are their volatile end products. This means attention should be paid to possible influencing factors such as dietary intake, drugs, smoking, environmental exposures, etc. The challenge in this respect is to balance constraints on subjects with the ease of use in clinic. As a general rule of thumb any factor influencing VOCs that is unbalanced between subject classes should be avoided as it may bias the discriminative signal (e.g. smoking when comparing obstructive lung disease patients with controls). With respect to other constraints, such as dietary, there is not sufficient evidence to suggest standardization improves sampling reliability.

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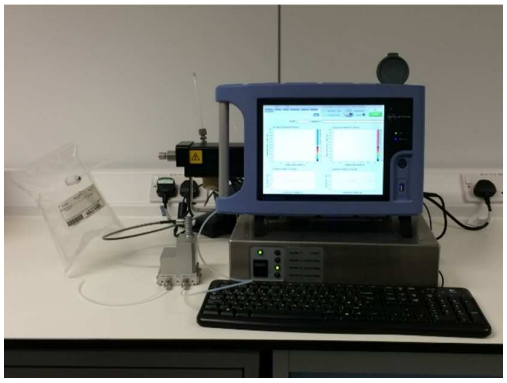
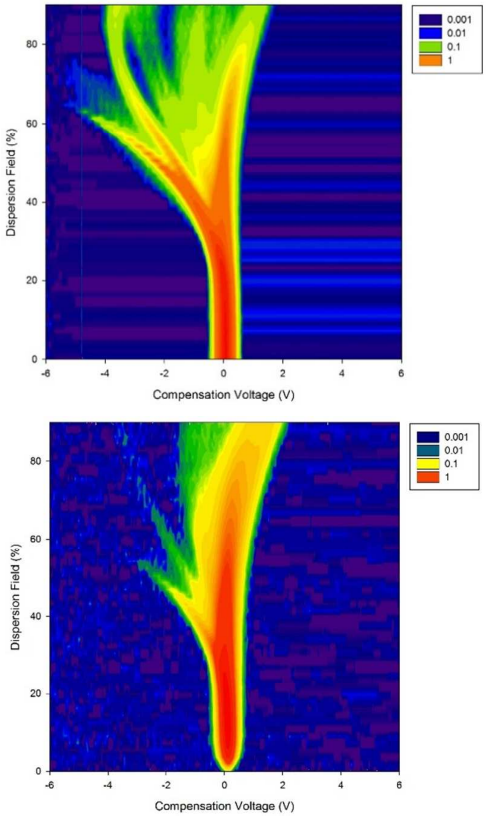


Figure 3: Owlstone Lonestar with Atlas sampling system analysing a breath sample

Figure 4 shows a typical chemical output from a healthy individual emanating from urine, stool and breath sample using FAIMS. As can be seen the chemical information of all of these samples differs immensely.



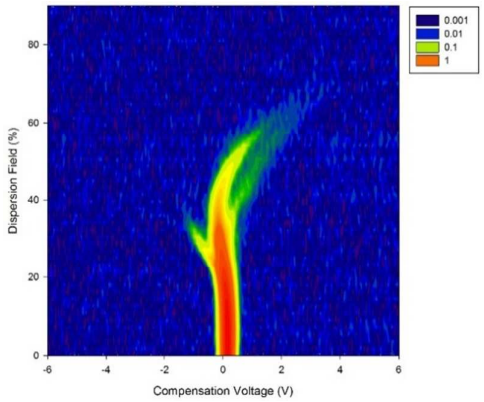


Figure 4: Typical FAIMS output for a (a) Stool, (b) Urine and (c) Breath sample.

Data processing

FAIMS produces high-dimensional data (in terms of the number of features/covariates measured per sample), rich with chemical information. Processing of such large datasets brings about its own challenges, which are shared with other omics approaches[7]. Most gas phase applications rely on the environment being stable, with the introduction of a single (or small number of) chemicals, that can easily be identified as foreign. In medicine, these chemical changes (either an addition, reduction or modulation in chemical composition) can be small when compared with a person's normal biological activity and in many cases the disease signals can be swamped by this "normal" signal. For decades, researchers have relied on a simple set of analytical tools, with principal component analysis and linear discriminant analysis being methods of choice. However, the high dimensional data coupled with the subtle chemical signals produced by the disease states make such methods, applied to the raw data, less effective or even causes them to fail outright.

One approach that has found favour with FAIMS is the use of compression algorithms to pre-process the data before feature identification and classification. Of these approaches, discrete wavelet transforms (as used with JPEG and Audio files) have been shown to be highly effective in reducing data complexity and enhancing feature quality, though other methods (such as Fourier Transforms) can also be effective. More modern methods such as sparse matrix decomposition techniques are likely to bring further improvements in this area.

After data reduction, identification of informative features is key. Approaches such as statistical hypothesis testing (e.g. Wilcoxon rank-sum test) have proved highly robust for identifying informative features. Other wrapper-based methods show promise, as they can identify interactions between individual features, and the future may be to deploy

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supervised feature learning techniques, which can both learn the structure underlying the data and also extract those features that are informative about outcome.

This can be followed by the use of a classification algorithm. Key is to identify which statistical and machine learning algorithms can best use these processed data to make predictions. Previous studies have used a range of modern classification methods to assess the sensitivity and specificity of a test, for example Random Forest, Sparse Logistic Regression and Support Vector machines. However, it is interesting to note that no one method wins out and it is highly dependent on the disease, making the task of model selection an important consideration. Ultimately any classifying algorithm should be externally tested by applying the algorithm to a newly-recruited population. Discriminating the effects that case-mix and incorrect coefficients have on the accuracy in the validation population are important to assess repeatability and transferability of the algorithms[8].

Medical Results

With health costs rising rapidly worldwide, clinicians are under pressure to explore newer and more economical diagnostic tools, especially if these are non-invasive. Thus the study of gases and volatile organic compounds (VOCs) is becoming increasingly attractive. It was first investigated in 1971[9] but then seemed to have lost its vogue. The development of improved analytical instruments and data-analytical algorithms have however resulted in an almost exponential increase in VOC diagnostics in the medical literature of late, reflecting its growing importance.

As mentioned, the role of VOC diagnostics in medicine is particularly attractive due to its non-invasive sampling – that is use of breath, urine, stool and even sweat. The origins of VOCs are threefold[9]. Firstly, exogenous compounds originate from external sources we inhale, ingest or absorb through our skin. These compounds are partly unwanted noise but may be valuable in studies of biological exposure and may reflect changes in resident microbiota relevant to disease development[10,11]. Secondly, local compounds originate at the site of the disease due to effects of the primary disease process such as aberrant cellular metabolism, cell death and inflammation. Finally, several volatiles have systemic origins caused by downstream effects of disease such as increased oxidative stress, catabolism and immune activation. As can be understood from this concept the relative contribution of each of these sources to the identified VOCs depend highly on the sample of choice and the disease of interest. This makes it an important choice in method development.

The analytical challenge is to reliably and reproducibly detect biomarkers of interest at low concentrations in a complex background. FAIMS has high potential to address this as the chemical fingerprint can be selectively screened for target compounds differentiating it from most eNose type applications. Furthermore the programmable nature of the technology means that after the initial biomarker validation steps the sensor can be selectively programmed to focus on those part of the spectrum that associate with disease. Combined with a diagnostic algorithm established during method development such applications can be easily used without requiring extensive training.

Various disease groups have been studied with FAIMS using different biological modalities. Table 1 lists these in detail within three broad categories – cancer, inflammation and infection.

These three overarching categories underpin most disease processes resulting in the shift of balance from health to disease. These processes are ubiquitous to most organs and tissues in the human body and illustrative of the potential applications of VOCs in clinical practice[10].

Cancer is effectively uncontrolled cell growth with loss of its regulatory check and repair mechanisms, typically caused by genetic changes inducing aberrant cellular metabolism. As these changes occur early in the disease process it opens possibilities for early screening of large at risk populations. This is supported by a pilot study showing promising detection rates of colorectal cancer from urine samples (sens/spec ; 88%/60%)[12]. To expand this an international multi-centre trial for early detection of lung cancer by FAIMS was recently initiated (http://www.owlstonenanotech.com/lucid).

Tissue inflammation is a hallmark of most disease processes. It follows a well-described molecular and chemical pathway making it an attractive candidate for non-invasive detection[13]. Firstly this means treatment could be commenced early thus halting further progression of inflammation as shown for Coeliac disease[19]. Secondly, identifying VOCs associated with inflammation can help to guide treatment decision by monitoring inflammatory activity, possibly even in the home[14]. Other potential applications include phenotyping of patients to stratify them to receiving the correct treatment as recently performed with FAIMS in patients with asthma[13].

Infections have been well studied since the days of Hippocrates, who realized that specific infections are associated with a particular scent. VOCs related to infection originate both from the metabolism of the pathogen (exogenous) and the response of the host (local and systemic)[14]. This is underlined by a study showing changes in bacterial populations could be tracked by FAIMS by using VOC profile changes[9].

This suggests VOCs can be used to diagnose infectious diseases and monitor treatment response[16]. This was recently shown by FAIMS in Hepatic encephalopathy, which is a result of toxins produced by gut bacteria not filtered effectively by the liver. Furthermore characterisation of microbial VOCs can provide a means to rapidly identify microbial dysbiosis associated with disease– as was shown

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for *C Difficile* infection[15]. In health this organism is a commensal (i.e. lives within us without causing harm). However, if this balance is disturbed and a shift to less favourable bacteria occurs, then *C Difficile* can become pathogenic.

Especially with infectious disease, the point of care nature of the FAIMS device can be a key advantage. After proper validation this could enable deployment not just in hospitals or primary care but also in third world countries. Realizing these goals is strongly supported by the appropriate technical developments.

Cancers	Numbers	Details	Samp.	Sens./ Spec.
Colorectal Cancer[12]	N=133; 83 colon 50 controls	Case control study of 83 with confirmed adenocarcinoma of the bowel	Urine	88/60 (a)
Pelvic radiation disease[16]	N=23	Longitudinal study – two groups High and low gut related toxicity following radiotherapy	Stool	90/90 (a)
Inflammation				
Inflammatory Bowel Disease (IBD)[17]	N=62; 48 with IBD 14 controls	Case cohort study; Comparison of FAIMS and metal oxide electronic nose	Urine	74/88 (a)
Inflammatory Bowel Disease (IBD)[18]	N = 76; 54 with IBD and 22 healthy controls	Case cohort matched study	Breath	74/75 (a)
Coeliac[19]	N = 47; 27 with histological confirmation of coeliac disease 20 controls with irritable bowel syndrome	Case cohort matched study	Urine	85/85 (b)
Bile acid diarrhoea[20]	N = 110 23 with bile acid diarrhoea	Case cohort study comparing two other	Urine	85/90 (a)
	42 with ulcerative colitis and 45 symptomatic controls	disease groups		
Eosinophilic airway inflammation[21]	N=52; 27 with eosinophilia	Case-control study using platform containing multiple techniques including FAIMS	Breath	Accuracy: 85%
Asthma/ COPD[24,25]	N = 78	Pilot study with DMS	Breath	No reported accuracy
Infection				
Clostridium Difficile[22]	N= 213 – 71 with C Diff positive by microbiological analysis	Case control involving a training, test and validation	Stool	92/86 (c)
Hepatic Encephalopathy (HE)[23]	N = 42; 22 with HE and 20 healthy controls	Case control study of 22 patients with various degree of HE (due to liver failure) compared with controls.	Breath	88/68 (b)

Table 1. Published studies applying FAIMS in a medical context. Data analysis method is indicated in the sens/spec column. (a) = wavelet transform, feature selection, then Linear/Fisher Discriminant Analysis ; (b) = wavelet transform, Wilcoxon rank-sum test for feature selection, then sparse logistic regression; (c) wavelet transform, Wilcoxon rank-sum test for feature selection, then Random Forest classifier

Future Developments

Likely future developments fall into a number of partially-overlapping categories, including improvements to the technology and associated data processing, increased ease-of-use and more extensive validation through larger clinical trials. Technical improvements will likely centre on increasing the strength of the electric field used in the instrument, allowing clearer separation of a wide range of compounds. At the same time, improvements in electronics should allow even lower concentrations of compounds to be detected. Improved ease-of-use, which will be necessary if FAIMS is to be a viable tool for widespread medical use, will require the overall size of current systems to be further reduced, a

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standardized sampling method to be introduced, and data-processing facilities to be incorporated into the instrument itself. It will also be necessary to change the current ionization source, radioactive Ni-63, to a non-radioactive alternative such as UV or corona discharge. These changes are currently being implemented and will allow for much larger clinical trials to take place. It will only be by conducting such larger clinical trials that the effectiveness of FAIMS for medical diagnostics will be properly determined.

Conclusions

With an aging population and a significant increase in the cost of healthcare, there is a strong demand to find simple, quick and inexpensive methods to diagnose and monitor human diseases. Non-invasive evaluation of metabolites by analysis of the gas phase biomarkers that emanate from human breath, urine, stool or sweat, shows considerable promise. Thus far a wide variety of approaches ranging from GC-MS to eNose have been evaluated. Field Asymmetric Ion Mobility Spectrometry (FAIMS) has the ability to bridge this gap between chemical analytical techniques and pattern recognition sensor based approaches because it combines high sensitivity with a reproducible detector in a point of care tool. We have shown the potential of FAIMS in a variety of medical applications including cancer, inflammatory diseases and infection. The coming years will see progress in terms of technical development and large clinical trials that will help identify how FAIMS can improve medical healthcare. Based on the broad available evidence it has the opportunity to revolutionize clinical pathways in primary and secondary care and even bring these tools into the home.

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