Moreno-Oyervides, A., Aguilera-Morillo, M. C., Larcher, F., Krozer, V. & Acedo, P. (2020). Advanced Statistical Techniques for Noninvasive Hyperglycemic States Detection in Mice Using Millimeter-Wave Spectroscopy. *IEEE Transactions on Terahertz Science and Technology*, 10(3), pp. 237–245.

DOI: 10.1109/tthz.2020.2967236

Advanced Statistical Techniques for Non-invasive Hyperglycemic States Detection in Mice Using Millimeter-Wave Spectroscopy

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***Abstract*— In this article, we discuss the use of advanced statistical techniques (functional data analysis) in millimeter-wave (mm-wave) spectroscopy for biomedical applications. We employ a W-band transmit-receive unit with reference channel to acquire the spectral data. The choice of the W-band is based on a trade-off between penetration through the skin providing an upper bound for the frequencies and spectral content across the band. The data obtained are processed using Functional Principal Component Logit Regression (FPCLoR), which enables to obtain a predictive model for sustained hyperglycemia, typically associated with diabetes. The predictions are based on the transmission data from non-invasive mm-wave spectrometer at W-band. We show that there exists a frequency range most suitable for identification, classification, and prediction of sustained hyperglycemia when evaluating the functional parameter of the FPCLoR model (β). This allows for the optimization of the spectroscopic instrument in the aim to obtain a compact and potential low-cost non-invasive instrument for hyperglycemia assessment. Furthermore, we also demonstrate that the statistical tools alleviate the problem of calibration, which is a serious obstacle in similar measurements at terahertz and IR frequencies.**

***Index Terms*— Functional Data Analysis (FDA), Functional Principal Components Logit Regression (FPCLoR), Millimeter-wave (mm-wave) spectroscopy, non-invasive diagnostics, sustained hyperglycemia, W-band reflectometer, W-band transmit/receive unit.**

This work was supported in part by the Spanish Ministry of Economy and Competitiveness under the Project TEC2017-86271-R, in part by the Instituto de Salud Carlos III (Spain) under Grant DTS17/00135, in part by the Ministerio de Economía y Competitividad, FEDER funds under Project MTM2017-88708-P, and in part by the Agencia Estatal de Investigación, Ministerio de Ciencia, Innovación y Universidades under Project IJCI-2017-34038. The work of Aldo Moreno-Oyervides was supported by the Consejo Nacional de Ciencia y Tecnología de México (CONACYT). *Corresponding author: Aldo Moreno-Oyervides.

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I. Introduction

Millimeter-wave (mm-wave) spectroscopy has received much attention for biomedical applications, due to its spectral resolution, high sensitivity, measurement speed, and richness in information contained in the acquired spectrum. Many biomedical applications have been addressed recently utilizing mm-wave spectroscopy [1]–[5], being diabetes detection one of the first applications envisaged for this technique. Microwave, mm-wave and terahertz (THz) spectroscopy have been proposed for Diabetes Mellitus (DM) diagnostic associated with instantaneous glucose level measurement [6]–[10] and sustained hyperglycemia states detection [11], both relevant to the diagnostic and patient control. However, in both cases, we are still far from an operational diagnostic based on these techniques that leverages the advantages identified for such a system, such as non-invasiveness, the use of nonionizing radiation, and the potential low cost of the developed system.

DM is a very complicated metabolic disorder affecting a great part of the world population, which is severely increased every year [12]–[16]. This metabolic disorder, which is characterized by the presence of high blood glucose content known as 'Hyperglycemia' [17], brings several and serious irreversible health consequences if it is not detected and treated on time: heart attacks, strokes, kidney failure, vision loss, etc. [18], [19]. The current medical procedures for diabetes control are mainly based on invasive technologies facing multiple disadvantages: high follow-up costs, painful in some cases, and disturbing in daily life, which explains somehow the unwillingness of people to completely follow medical recommendations in diabetes care. Therefore, there is a worldwide effort to develop new technologies that are able to diagnose and monitor diabetes non-invasively (or minimally invasive), improving the existing medical procedures, specially using spectroscopic techniques but with limited success due to the lack of accuracy or feasibility of the proposed spectroscopic technique [20]–[24]. Furthermore, the measure of free glucose in the blood as the diagnostic parameter for diabetes also has its drawbacks, as blood glucose level is strongly conditioned by many physiological processes, such as eating, exercise or the related ones to strong emotions. Hence, new non-invasive techniques that reduce the influence of external factors enhancing the diagnosis and monitoring of diabetes are needed.

An important indicator of DM is hyperglycemia, generally caused by DM and having a strong impact on the physiological

functionality of the organism. The formation of Advanced Glycation End-products (AGEs) associated with the sustained high-glucose concentration in blood is responsible for the most relevant complications associated with DM. An early and non-invasive diagnostic for such sustained hyperglycemic states is crucial, not only for diabetes diagnostics thus facilitating an early intervention to regulate the carbohydrate metabolism but also for metabolomic control and supervision of the patients.

This article proposes a mm-wave reflect/transmit setup using a multiplied source and subharmonic Schottky diode receivers and appropriate statistical spectroscopy techniques to arrive at a diagnostic tool for non-invasive control of sustained hyperglycemia. The focus of the paper is on the discussion of the importance of using adequate statistical techniques to process the spectral data in mm-wave spectroscopy to develop biomedically relevant diagnostic systems, as for example, in DM diagnostic.

Until now, multivariate statistical techniques have been widely used for data analysis and statistical modeling in spectroscopic applications (e.g. [25]–[29]), and for optimization of the frequency spectrum used for interrogation (e.g. [30], [31]). In biomedical spectroscopic applications, the acquired data correspond to complicated spectra with overlapping responses from many different constituents of the biomedical sample. This overlap of spectral responses prevents the isolation of a specific component within the sample and perform spectral classification. In addition, other factors adversely affecting the spectral classification, such as environmental influences, instrument noise, and interferences by physiological processes, have an impact on the spectral data and necessitate the systematic analysis of a set of discrete and independent variables. The acquired spectral data might become very large and lead to dimensionality problems, hindering the data processing, and requiring dedicated statistical analysis. The Functional Data Analysis (FDA) [32] is currently employed in biomedical applications based on spectroscopic techniques [33], [34] since it provides a set of more suitable and powerful statistical tools for the processing and analysis of the spectral information.

We demonstrate in this article a spectrometer working in the W-band (75-110 GHz) with reflect and transmit measurement capabilities using a nonspecific approach. In this way, the proposed technique allows us to discriminate the metabolic animal condition (normoglycemic or hyperglycemic) without the need to isolate or quantify the specific response of a single component or metabolite. To achieve this, we employ FDA to obtain a continuous representation (function) from each spectrum measured at discrete frequencies within the W-band. Continuous representation of the spectrum using FDA allows for better flexibility for high-dimensional data and longitudinal data analysis, as well as improves the spectral variability analysis.

In our spectral analysis, we apply the Functional Principal Component Logit Regression (FPCLoR) [37] to obtain a predictive model as a diagnostic tool for hyperglycemia in the animal models. We study qualitatively and quantitatively how the obtained spectral response characterizes the hyperglycemia condition in the animal models by interpreting the FPCLoR model. Finally, we demonstrate that the frequency bandwidth can be halved using the FPCLoR model when choosing the main contributing frequencies to hyperglycemia discrimination. These results show the potential of the FDA establishing a feedback to the W-band spectral results and the spectroscopic analysis tool presented here.

The article is organized as follows. In section II, we describe the spectroscopic instrument and methods of sample preparation. In section III, we study and discuss the data acquisition and data processing algorithms, and in section IV, we discuss the achieved results. Finally, the article finishes with the conclusions and outlook.

II. Materials and Methods

A. Millimeter wave spectroscopy instrument

The spectral interrogation for the non-invasive assessment of sustained hyperglycemia was carried out by a spectrometer operating across the full W-band with steps of 1.5 GHz. As shown in Fig.1, generators deliver a swept signal in the Ku-band (12.5-18.5 GHz in steps of 0.25 GHz) with 0.3 MHz frequency difference between the two signals. One signal generator is connected to an Active Frequency Sixtupler (MULT), resulting in a frequency sweep within the W-band. The MULT is realized in a waveguide housing and exhibits a WR10 waveguide output. The output signal from the MULT is fed to a dual-directional coupler. The coupled arms of the coupler define the reference and reflect channels, respectively, with subharmonic mixer receivers at each coupled port. The thru branch of the coupler is fed into a waveguide probe and device under test (DUT). The transmission path is measured with equivalent subharmonic mixer receivers, as in the reference and reflect ports. The outputs of the subharmonic receivers deliver an intermediate frequency of IF = 1.8 MHz and are connected to a data acquisition unit (Handyscope HS4-10, TiePie engineering, Sneek, Netherlands), which digitizes the measured signals with a sampling rate of 10 MHz. Finally, all the sampled signals are filtered and processed using LabVIEW software.

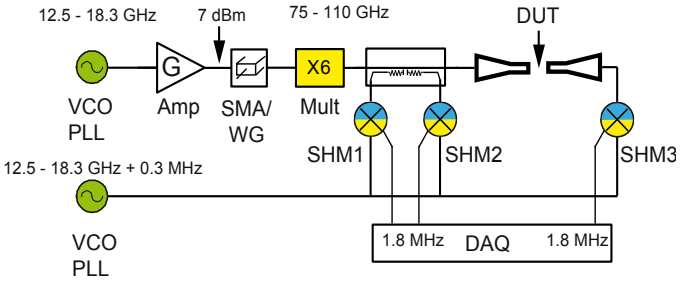

Fig. 1. Simplified block diagram of the set-up. See text for details.

More details of the spectrometer already reported can be found in [11], [38]. Fig. 2 shows a photograph of the mm-wave instrument identifying all the components mentioned above.

In the present article, we exclusively employ transmission amplitude measurements, even though phase results are also available, as the amplitude values are sufficient for the FPCLoR analysis. The output power of the signal generator was adjusted

to obtain a flat frequency response at the transmission port without the biological sample. No previous calibration is required for the amplitude data. These aspects enormously simplified the diagnostic system and allows for further low cost and compact implementations.

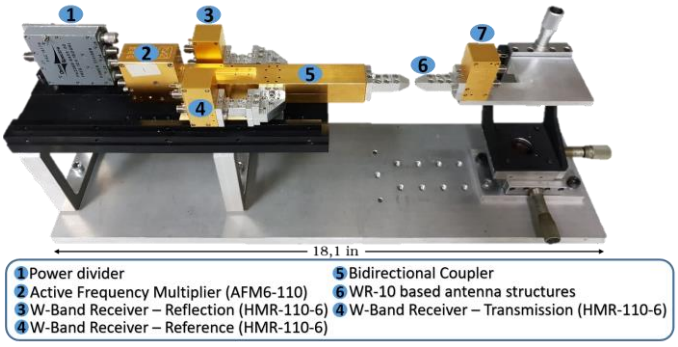

Fig. 2. Mm-wave spectroscopic instrument for the non-invasive sustained hyperglycemia assessment.

B. Experimental protocol

The mm-wave spectroscopic measurements were performed at the Centro de Investigaciones Energéticas, Medioambientales, y Tecnológicas (CIEMAT) Laboratory Animals Facility (Spanish registration number 28079-21 A), which provided the animal models. The animals were not subjected to special treatments before the mm-wave measurements. Mice hair were regularly cut to ensure and facilitate the positioning of the probes but were not totally shaved. Insensitivity of the measured results to hair skin in mice was proven earlier in [7]. Prior to the measurement process, each mouse was anesthetized to prevent movement and self-harm risks during the measurements. Two anesthesia methods were followed to carefully avoid the impact of anesthesia treatment. In experiment A, the anesthesia was administered by injection using standard rodent anesthesia (ketamine/medetomidine). Then, for experiment B, the mice were anesthetized by inhalation using isoflurane mixed with oxygen, which reduces the induction time and is less harmful to animals.

As shown in Fig. 3, mice were assessed one by one, and the spectral interrogation was performed directly on a fold of the skin on the back of each mouse. The probes of the spectrometer instrument were two previously aligned straight cuts of a WR10 waveguide tapered on the outside to facilitate the skinfold. The separation between the straight cuts was fixed to hold the skinfold without harming the animal. The measuring process takes around 45 seconds and the anesthetic gas was continuously supplied to the animal via a mask during the whole period in experiment B. The measurement time is mainly limited by the control electronics and not by the mm-wave instrument.

All experimental procedures were carried out according to European and Spanish laws and regulations (European convention ETS 1 2 3, about the use and protection of vertebrate mammals used in experimentation and other scientific purposes, Directive 2010/63/UE and Spanish Law 6/2013, and R.D. 53/2013 about the protection and use of animals in scientific research). Procedures were approved by the Animal Experimentation Ethical Committee of the CIEMAT according to all external and internal bio-safety and bio-ethics guidelines, and by Spanish competent authority with registered number PROEX 176/15.

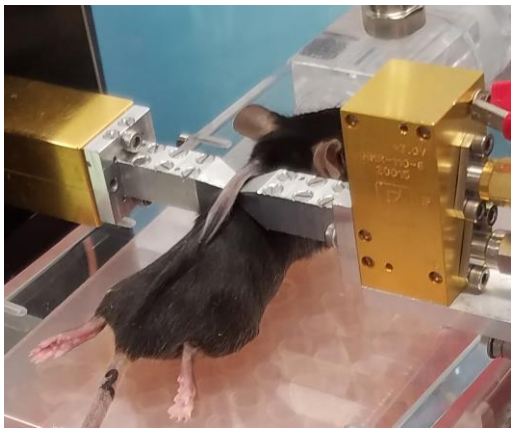

Fig. 3. Photograph taken during the measuring process.

C. Sample population

Two experiments were conducted with sixteen months elapsed between the first and second experiment, and a different sample of mice for each experiment referred to as “A” and “B”. In both experiments, the sample of mice included animals with normoglycemic and hyperglycemic conditions. Healthy mice of different strains with an expected blood glucose level of 100 mg/dl were considered as normoglycemic animals. Within the hyperglycemic cases, two different types of hyperglycemia were considered: obese mice due to a genetic mutation that causes a deficiency of leptin (Lep^{ob}/ Lep^{ob}) and diabetic mice due to a genetic mutation that causes insulin resistance (Lep^{db}/ Lep^{db}) [39]. The expected glucose level for the hyperglycemic cases is above 150 mg/dl and 250 mg/dl for the obese mice and the diabetic mice, respectively. TABLE I summarizes the sample of mice measured at each experiment.

All animals were purchased from Elevage-Janvier (France) and housed individually in pathogen-free conditions at CIEMAT.

TABLE I
SAMPLES POPULATION DESCRIPTION

Experiment	Condition	Type	Mean glucose level	Label	Quantity
A	Normoglycemia	-	136 mg/dl	Healthy	10
	Hyperglycemia	Leptin-deficient	180 mg/dl	Obese	5
	Hyperglycemia	Insulin-resistant	500 mg/dl	Diabetic	5
B	Normoglycemia	-	144 mg/dl	Healthy	18
	Hyperglycemia	Leptin-deficient	245 mg/dl	Obese	9
	Hyperglycemia	Insulin-resistant	380 mg/dl	Diabetic	6

III. DATA PROCESSING AND STATISTICAL ANALYSIS

As described in section II, the spectroscopic measurement provides a 1-D array (vector) whose entries correspond to a finite set of observed amplitudes for each frequency. This vector is then transformed into a continuous function of frequency, which allows the application of FDA [32].

A. Functional data approximation

Measurements are usually affected by, systematic errors, noise and/or other external interferences. Therefore, data is assumed to be smooth but observed with an associated error, in our case:

$$x_{ik} = x_i(f_{ik}) + \epsilon_{ik}, k = 1, \dots, m, i = 1, \dots, n, \qquad (1)$$

where x_{ik} is the i-th discrete sample path (raw spectra), $x_i(f_{ik})$ is a smooth function observed at the frequency points f_{ik} and ϵ_{ik} is an error term representing noise, with n being the sample size (number of assessed mice) and m the number of frequency points. Then, the function is approximated by assuming that the sample paths belong to a finite dimensional space generated by an orthogonal basis $\{\phi_1(f), \dots, \phi_p(f)\}$, so that

$$x_i(f) = \sum_{j=1}^{p} a_{ij}\phi_j(f),\ f \in F, i = 1, \dots, n, \qquad (2)$$

where $x_i(f)$ is the estimated curve, a_{ij} are the basis coefficients, $\phi_j(f)$ are the basis functions, and F is the observation interval (frequency interval). Since our data is smooth but observed with some noise, a basis of smooth functions must be considered. Cubic B-splines basis generates the space of splines of degree 3, defined as curves consisting of piecewise polynomial of degree 3, that join up smoothly in a set of nodes with continuity in their derivatives up to order 2 [40]. Regression splines and P-splines can be used to estimate the basis coefficients with least squares criterion and differing by the control of the degree of smoothness of the fitted curves. P-splines add a penalty term in the least square's equation so that the lack of smoothness in the curves is controlled by a smoothing parameter λ [41]. Obviously, the degree of smoothness in the fitted curves influences the statistical analysis and the data interpretation. Therefore, the researcher, according to the goal of the experiment and the nature of the measure, should define such adjustment. Fig. 4 shows two different approximations by using regression splines, defined on 17 not equally spaced nodes (at the top) and P-splines defined on 17 equally spaced nodes with $\lambda = 0.11$ (at the bottom). For each approximation, the fitted curves for a normoglycemic (left panel) and a hyperglycemic (right panel) case are shown in Fig. 4. Both approximations will be discussed in the results section.

B. Functional Principal Component Analysis

A Functional Principal Component Analysis (FPCA) [42] was applied to the measured W-band data to explore and highlight variability among the estimated functional spectra, determining a set of uncorrelated functions called Functional Principal Components (FPCs). The principal components are obtained as uncorrelated generalized linear combinations of the

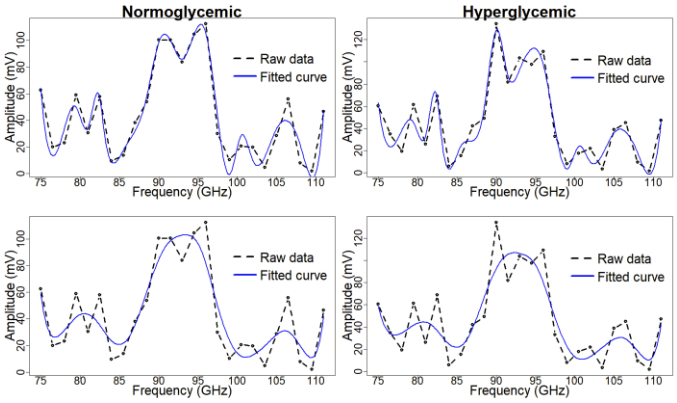

Fig. 4. Estimated and measured transmission amplitude for a normoglycemic (left panel) and a hyperglycemic mouse (right panel) by using two different approximations: regression splines (at the top) defined on 17 not equally spaced nodes and P-splines (at the bottom) defined on 17 equally spaced nodes with $\lambda = 0.11$.

sample curves with maximum variance. In general, the j-th principal component scores are given by

$$\xi_{ij} = \int_F x_i(f)w_j(f)df, \qquad i = 1, \dots, n, \qquad (3)$$

with w_j being the weight functions or loadings obtained by solving the following maximization problem, under the orthonormality constraints

$$\begin{cases} \max_{w} var\left[\int_F x_i(f)w(f)df\right] \\ s.t. \|f\|^2 \text{ and } \int_T w_j(f)w_l(f)df = 0, \qquad \forall\, j < l. \end{cases} \qquad (4)$$

For more details see [32]. The advantages of working with FPCs are reduced dimensionality of the statistical problem and avoidance of multicollinearity problems in the regression analysis.

C. Functional Principal Component Logit Regression

The detection of sustained hyperglycemia has been addressed as a classification problem with a binary response: normoglycemia or hyperglycemia. Therefore, we applied functional logit regression [43] to obtain a predictive model. The logit regression is widely used in social and medical applications, modeling qualitative variables by a set of independent variables as predictors. The Functional Logit model (FLoR) is the extension of the logit regression to the FDA context, but in this case, the predictor is a functional variable. The FLoR model is formulated as follows:

$$y_i = \pi_i + \epsilon_i, \qquad i = 1, \dots, n \qquad (5)$$

where $y_i \in \{0,1\}$, $i = 1, \dots, n$, is the value of the response variable associated to the i-th observation of the functional predictor $x_i(f)$, being $0 \equiv$ normoglycemia and $1 \equiv$ hyperglycemia, ϵ_i are the independent errors with zero mean

and $\pi_i = P[Y = 1|\{x_i(f): f \in F\}]$ is the expectation of the response variable given by

$$\pi_i = \frac{exp\{\alpha + \int_F x_i(f)\,\beta(f)df\}}{1 + exp\{\alpha + \int_F x_i(f)\,\beta(f)df\}}, \quad i = 1, \dots, n. \tag{6}$$

As it can be seen in (6), π_i is modelled by a real parameter α and a functional parameter $\beta(f)$. Equivalently, (6) can be rewritten in terms of the logit transformation as follows:

$$l_i = ln\left[\frac{\pi_i}{1-\pi_i}\right] = \alpha + \int_F x_i(f)\,\beta(f)df, \quad i = 1, \dots, n. \tag{7}$$

In order to solve the estimation problems in the FLoR model a set of FPCs can be used as predictor variables [37]. Then the FLoR model can be expressed in matrix form in terms of a reduced set of q principal components (FPCLoR) as follows:

$$L = \alpha + \Gamma\gamma, \tag{8}$$

with $\Gamma = (\xi_{ij})_{nxq}$ being the matrix comprising the columns of the first q principal components and γ the vector of the model coefficients. Finally, by assuming the basis representation of the functional parameter $\beta(f)$ and the weight functions $w_j(f)$, $j = 1, \dots, p$, the functional parameter of the FLoR model is estimated by $\beta = \mathbf{M}_{(pxq)}\gamma_{(qx1)}$, where $\beta = (\beta_1, \dots, \beta_p)'$ is the vector of basis coefficients of $\beta(f)$ and $\mathbf{M}$ is a matrix whose columns contain the vectors of basis coefficients of the weight functions associated with the first q principal components [37].

A significant additional contribution of the FLoR is the interpretation of $\beta(f)$. This function represents the relation between the response variable and the functional predictor, and it can be interpreted in terms of the odds ratio [44]. Let us consider l_i the logit transformation of a sample function $x_i(f)$ and l_i^* a resulting scaled logit transformation of a sample function $x_i^*(f)$ scaled by the factor K within a frequency bandwidth $[f_0, f_{0+h}] \subseteq F$. Then, the odds ratio for l_i and l_i^*, considering (7) will be

$$exp(l_i^* - l_i) = exp\left(K \int_{f_0}^{f_{0+h}} \beta(f)df\right). \tag{9}$$

This means that a constant increment in K units in a fixed interval for $x(f)$ increases the odds of $y = 1$ against $y = 0$ by a factor of the same magnitude K. This kind of interpretation is very useful to understand the relation between the spectral response, measured by the spectroscopic system and the achieved classification. This interpretation provides not only information (in the quantitative sense) about the aimed discrimination but also it can be useful to identify which interrogation frequency intervals are more relevant for such a discrimination. This approach will be illustrated in the results section by interpreting the FPCLoR for the sustained hyperglycemia detection in animal models.

IV. Results and Discussion

This section focuses on the robustness and prediction of the diagnostic tool utilizing W-band measured spectra. First, sample B is used for a multi-test analysis to study the performance and robustness of the fitted model for hyperglycemia discrimination. In this study, the results obtained from both approximations, regression splines and P-splines, will be compared. Then, in a second subsection, the diagnostic tool will be validated by testing the prediction capability of the fitted model on the sample A. Additionally, the FPCLoR model is analyzed by interpreting the functional parameter. All the data processing and analysis were performed using the statistical software R project [45], and the *fda* package [46].

An important observation of the diagnostic tool is that the measured amplitude of the transmitted skinfold signals is sufficient for accurate analysis. Even though the phase of the signal is also acquired by the spectroscopy system, results did not improve when taking phase into account. Furthermore, there is no need to preprocess or calibrate the amplitude data.

A. Multi-test analysis: performance and robustness of the diagnostic tool

The functional data obtained from sample B was employed for a multi-test analysis. As reported in TABLE I, the functional data contains 33 observations. The training sample consisted of 80% of the functional data and the remaining 20% were assigned as a test sample. The observations for each group were selected randomly preserving the original proportion of both classes within the global group: 54% of the cases are normoglycemic and 46% are hyperglycemic. Then, a FPCLoR model was fitted from the training sample, and the test sample was used to assess the prediction of the model. This process was repeated 100 times, and every time the performance of the predictive model was evaluated measuring four validation parameters: the Area Under ROC Curve (AUC), True Positive Rate (TPR), True Negative Rate (TNR) and Correct Classification Rate (CCR). The TPR and TNR, also known as sensitivity and specificity, respectively, are commonly used in medical diagnostics [47]. The TPR, TNR and CCR values are estimated taking into account the confusion matrix, shown in TABLE II, as follows:

$$TPR = \frac{TP}{TP+FN}, \quad TNR = \frac{TN}{TN+FP}, \quad CCR = \frac{TP+TN}{TP+TN+FP+FN}$$

TABLE II
CONFUSION MATRIX

		True condition	
		Hyperglycemia	**Normoglycemia**
Predicted condition	**Hyperglycemia**	Hyperglycemia correctly classified (True Positive)	Normoglycemia misclassified (False Positive)
	Normoglycemia	Hyperglycemia misclassified (False Negative)	Normoglycemia correctly classified (True Negative)

The Receiver Operating Characteristic (ROC) curve shows the inverse relationship between the sensitivity and the specificity (sensitivity vs 1-specificity) varying the diagnostic criterion (the defined value to assign $y = 1$) of the test, and the

AUC, which can be estimated by numerical integration methods, provides an effective measure of the diagnostic accuracy of the predictive model. TABLE III summarizes the results of the multi-test analysis for both approximations: regression splines and P-splines (see Fig.4).

Comparing both cases, we can see that the mean value of AUC is slightly higher for the model based on regression splines than P-splines. However, for both cases, this value is above 0.95 (excellent discrimination capability) and very robust with a standard deviation under 0.02. This indicates that the FPCLoR model clearly discriminates the inter-condition (normoglycemia and hyperglycemia) variability captured by the spectroscopic instrument. As shown in TABLE III, the predictive capabilities for both cases are very good with a mean value of CCR above 80%, being 10% higher for the case of regression splines. These results suggest that the FPCLoR model obtained from regression splines is the best in terms of the prediction capabilities. Nevertheless, as mentioned above, a significant advantage of working with the FPCLoR is the interpretation of the estimated functional parameter $\beta(f)$, which offers relevant information to improve the diagnostic tool. Here, we can emphasize that the lack of smoothness in functional data is reflected in the estimated functional parameter. Fig. 5 shows one of the functional parameters estimated for both approaches, based on regression splines (left panel) and P-splines (right panel). The functional parameter estimated from the regression splines exhibits strong oscillation versus frequency making its interpretation difficult. Considering a noisy function, as the obtained from the regression splines, it can be seen in (9) that the frequency domain must be divided in several subintervals so that the integral of the beta function does not tends to zero, which implies an estimated odds ratio close to one. This minimizes the contribution of the frequencies for discrimination and forces to consider very short frequency intervals, losing a lot of information. On the other hand, a smoother functional parameter is obtained by the P-spline approach. Therefore, in the next subsection, we will work with the P-splines since the resulting functional parameter offers a much better interpretation of the FPCLoR model and both provide excellent multi-test results.

TABLE IIII
SUMMARY OF THE MULTI-TEST ANALYSIS

Parameter	Regression splines		P-splines	
	Mean	Std. Dev.	Mean	Std. Dev.
AUC	0.99	0.01	0.96	0.02
CCR	0.92	0.08	0.82	0.11
TPR	0.91	0.18	0.74	0.23
TNR	0.93	0.11	0.87	0.18

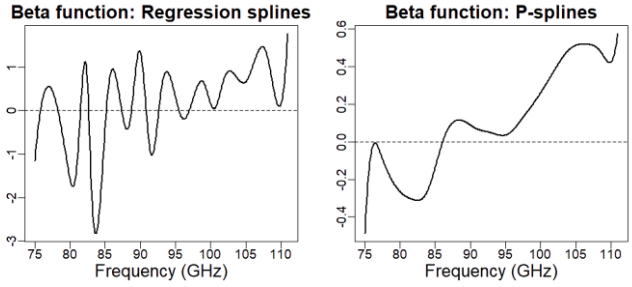

Fig. 5. Functional parameter $\beta(t)$ estimated for FPCLoR on the functional data approximated by regression splines and P-splines (left and the right, respectively).

B. *Validation and analysis of the diagnostic tool.*

The multi-test analysis showed that the diagnostic tool allows for correct diagnosis (detection) of hyperglycemia in animal models with excellent and very robust results. Then, sample A, measured at a second experiment, was used to validate the consistency of the diagnostic tool to discriminate the condition in a new sample. Once again, a FPCLoR model was fitted using the 33 mice from sample B as training sample, and sample A was the test sample. As reported in TABLE I, sample A consists of 20 mice with hyperglycemia and normoglycemia proportionately distributed. Functional data from both, sample A and sample B were estimated by using P-splines defined on 17 equally spaced points in the spectrum, as shown in Fig.4 (bottom). The ROC curve corresponding to the fitted model is shown in Fig. 6 with the AUC = 0.95. Testing the prediction capabilities of the fitted FPCLoR model on the test sample, we obtain a 100% on the new observations correctly classified (CCR). These results validate the consistency of the measured spectral response for sustained hyperglycemia, and consequently, supports the reliability of the spectroscopy instrument.

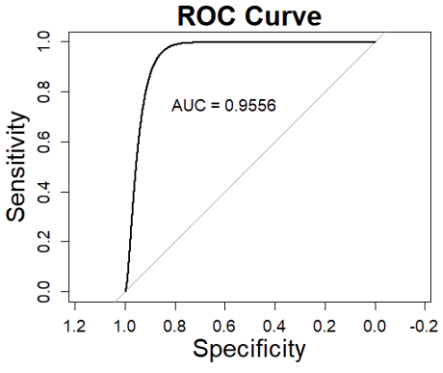

Fig. 6. ROC Curve estimated for the FPCLoR model.

Finally, the functional parameter for the sustained hyperglycemia discrimination, obtained from the FPCLoR model, is shown in Fig. 7. As a first observation, we notice that the beta function varies from negative to positive values versus frequency with a zero crossing at 86 GHz, indicated by a red line. The frequency interval is then subdivided into two sections, which inversely relate the spectral response and the hyperglycemia. To illustrate this, we will interpret the odds ratio for both frequency intervals considering a constant increase in the measured transmission amplitude of 0.3mV ($K = 0.3$) for the spectral response. The estimated odds ratio for both frequency intervals is presented in (10) and indicate that such a constant increment in the transmitted amplitude for frequencies under 86 GHz reduce the odds of being diabetic to one half, in contrast to frequencies above 86 GHz, where the odds is six-fold. In this way, we are able to measure the impact of a change in the spectral response on the diagnosis, enabling for further development of a quantified diagnostic measure for accurate diagnosis in management of diabetes

$$OR_{75-86}^{0.3} = 0.516 \quad OR_{86-111}^{0.3} = 6.52. \tag{10}$$

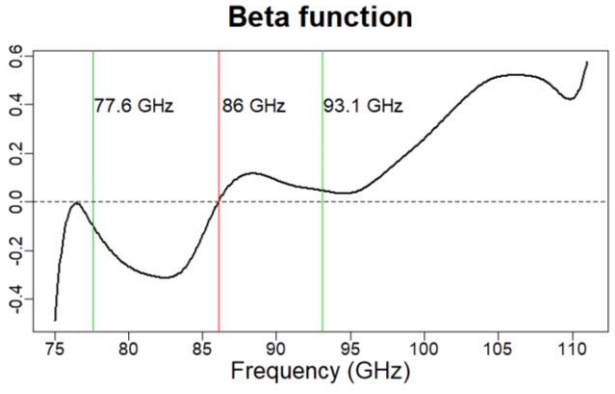

Fig. 7. Discriminating function $\beta(t)$ for sustained hyperglycemia detection of the FPCLoR model. The red line indicates a sign change for the function at the frequency of 86 GHz. The green lines indicate the frequencies, chosen arbitrarily, as the lower and upper limits for a reduced frequency band.

Additionally, to show the potential of the statistical approach, we reduce the frequency band, keeping the inflection point of the functional parameter (~ 86 GHz) within the band, with the lower and upper frequency limit chosen arbitrarily to be 78 GHz and 93 GHz, respectively, indicated by green lines in Fig. 7. The frequency range was delimited around the frequency in which the functional parameter crosses zero since that frequency interval provides two regions which relates strongly and inversely the spectral response to the hyperglycemia condition, enhancing the discrimination. The reduction of the frequency range represents a drastic improvement in measurement time, if one can assume to use the same frequency step. It has also an important impact of instrument complexity. However, since less frequencies are considered, less information is provided to the regression model, and that may affect the discrimination. Therefore, the discrimination of sustained hyperglycemia was evaluated on the new measurement span (78-93GHz). As before, a FPCLoR model was fitted for the resulting set of functional data estimated from the reduced spectra corresponding to sample B. Then, the prediction capability of the FPCLoR model was tested on sample A. For both data sets, functional data were approximated by P-splines defined on 11 equally spaced spectral points with $\lambda = 0.528$. The validation parameters of the fitted FPCLoR model for the selected frequency interval are AUC = 1, CCR = 0.95, TPR = 0.90, and TNR = 1. This represents an improvement in discrimination as compared to TABLE III. This improvement is attributed to the fact that the major contribution to the discrimination in our case originates from this frequency interval and the remaining frequency interval is essentially contributing with potential errors to the overall analysis.

V. Conclusions

This article demonstrates the suitability of mm-wave spectroscopy in the W-band for hyperglycemia discrimination using advanced statistical methods. The article also shows that the model developed here is predictive and provides excellent results across several independent tests. The results obtained for sustained hyperglycemia discrimination and prediction, which is strongly related to diabetes condition, were based on spectral data from the transmission reflection setup covering the full W-band.

The spectral data employed in this work are based on transmission amplitude data only, demonstrating that the amplitude spectrum contains the major information, leading to the conclusion that transmission through the skinfold is important. An advantage of this technique is its inherent insensitivity to skin reflectivity. Moreover, no previous calibration by a reference response from spectrometer has to be performed to the measured amplitude prior the statistical analysis. These results simplify the overall spectroscopy system resulting in a simple transmission type spectrometer with limited frequency bandwidth of operation. We also show that interpreting the FPCLoR model allows to detect the most contributing frequencies for discrimination so that spectral interrogation can be optimized by selecting a substantially narrower frequency band enough for discrimination.

A major contribution lies in the advanced statistical techniques applied to mm-wave spectroscopy data. We have used a set of measured spectra representative of different glycemic states in animal models. Normoglycemic mice with an expected blood glucose level ~ 100 mg/dl and mice suffering sustained hyperglycemia with chronically elevated levels of glucose in blood >150 mg/dl, were non-invasively tested by the mm-wave instrument. The transmitted amplitude data were evaluated applying FPCLoR obtaining a predictive model for sustained hyperglycemia, typically associated with diabetes. The analysis was carried out combining a non-specific approach and FDA. A multi-test analysis, with 100 iterations, was performed on a sample of 33 mice (18 normoglycemic cases and 15 hyperglycemic cases) studying two different approaches for the approximation of the functional data: regression splines and P-splines. The mean values for both approaches in an area under the ROC curve AUC > 0.95 and in a correct classification rate CCR > 80%. The multi-test analysis

shows that discrimination of hyperglycemia was slightly better using regression splines, but the lack of smoothness in the approximation of the functional data makes it difficult to interpret the fitted model. Considering the roughness of the approximation for functional data, we were able to reduce the spectral frequency range by almost one half, with only a 5% penalty, as the CCR decreased from 100% to 95% in the validated predictive model. These results indicate the great applicability of combining the mm-wave spectroscopy with a non-specific approach for new non-invasive diagnostic tools, and the potential of the FDA developing spectroscopic diagnostic tools.

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In 2002 he was appointed as Assistant Professor by Universidad Carlos III de Madrid where he continued with the development of scientific instrumentation systems for fusion plasma diagnostics and biomedical applications, leading national projects and contracts on these fields. Starting 2009 his research interests focused on the development of multimode laser sources (Optical Frequency combs) and their applications in photonic signal synthesis with the development of several architectures for the generation, detection and processing of THz signals, leading several research projects and contracts in this field. In 2014 he demonstrates with his team one of the first implementations of Dual-Optical Frequency Comb architectures based on electro-optical modulators and its use in dispersion spectroscopy and other applications beyond spectroscopy, which has made him an international reference in this type of optical sources.

In 2013 he intensifies his activity in biomedical applications including the development of diagnostics such as an instrument for the early detection of vascularization in engraftments with applications in tissue engineering, and a non-invasive system for the measurement of sustained states of hyperglycemia. Nowadays his interests continue to focus on the development of scientific instrumentation systems, sensors and circuits, and their application in fields such as bioengineering and environmental measurements.

Prof. Acedo has published more than 130 articles in high impact journals and communications to International Congresses, including invited conferences and seminars. He has also directed 9 research projects (European, national and regional funding) and has participated in another 31 (five of them EU funded). Finally, it is worth mentioning his leadership in knowledge transfer activities, where he has been IP in 12 contracts with companies (INDRA, AIRBUS D&S, LWL, SEAC, ...) and participated in another 6; works that have led to various patents. He was also the promoter of the creation and development of the spin-off Luz WaveLabs (www.luzwavelabs.com), which exploits most of his developments in the photonic synthesis of THz and pulsed optical sources.