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Functional Mixed-Effects Modeling Of Longitudinal Duchenne Muscular Dystrophy Electrical Impedance Myography Data Using State-Space Approach

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Abstract

Objective: Electrical impedance myography (EIM) is a quantitative and objective tool to evaluate muscle status. EIM offers the possibility to replace conventional physical functioning scores or quality of life measures which depend on patient cooperation and mood.

Methods: Here, we propose a functional mixed-effects model using a state-space approach to describe the response trajectories of EIM data measured on 16 boys with Duchenne muscular dystrophy (DMD) and 12 healthy controls, both groups measured over a period of 2 years. The modeling framework presented imposes a smoothing spline structure on EIM data collected at each visit and taking into account of within subject correlations of these curves along the longitudinal measurements. The modeling framework is recast in a state-space approach, thereby allowing for the employment of computationally efficient diffuse Kalman filtering and smoothing

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CONFLICT OF INTEREST

Dr. Rutkove has equity in, and serves as a consultant and scientific advisor to Myolex, Inc., a company that designs impedance devices for clinical and research use; he is also a member of the company's Board of Directors. The company also has an option to license patented impedance technology of which Dr. Rutkove is named as an inventor. Dr. Sanchez is named as an inventor on several patent application in the field of electrical impedance. Dr. Sanchez serves as a consultant to Maxim Integrated, Inc., and Impedimed, Inc., two companies that develop impedance devices for consumer electronics, clinical and research use. This study, however, did not employ any relevant company or patented technology.

algorithms for the model estimation as well as the estimates of the posterior variance-covariance matrix for the construction of the Bayesian 95% confidence bands.

Results: The proposed model allows us to simultaneously adjust for baseline variables, differentiate the longitudinal changes in the smooth functional response and estimate the subject and subject-time specific deviations from the population-averaged response curves. The code is made publicly available in the supplementary material.

Significance: The modeling approach presented will potentially enhance EIM capability to serve as a biomarker for testing therapeutic efficacy in DMD and other clinical trials.

Keywords

Functional data; mixed-effects models; electrical impedance myography; Kalman filtering; smoothing splines

I. INTRODUCTION

DUCHENNE muscular dystrophy (DMD) affects approximately 1 in every 3,500 live male births [1]. Although one therapy, corticosteroids, has been found to be mildly effective in slowing progression, the disease is still universally fatal with death usually by the age of 30 years. However, given an improved understanding of disease pathogenesis [2], a variety of promising new therapies, including antisense oligonucleotide and gene therapy are being tested [3]. Thus, accurate biomarkers to disease progression and detect therapeutic effect are being sought.

Conventional clinical outcome measures such as the six-minute walk distance (6MWD) test and North Star Ambulatory Assessment (NSAA) can be subjective and limited by the patient's degree of effort. Moreover, these outcome measures cannot be accurately performed in the very young or severely affected older patients [4]. Muscle biopsy can provide detailed histological assessment of muscle tissue, however, it is impractical for longitudinal studies on disease progression and inconvenient to perform in the pediatric population. Magnetic resonance imaging can assess muscle architecture, fatty infiltration and muscle oxidative metabolism [5]; however, it is expensive and can be challenging to perform in children. Finally, ultrasound is user friendly, relatively inexpensive, and can be performed at the bedside [6], [7]. However, it is difficult to quantify the muscle signal in a consistent fashion, alterations in probe angle and pressure applied distort image quality, and only small regions of the muscle may be evaluated at any one time.

Electrical impedance myography (EIM) is another method available to assess the progression of neuromuscular diseases such as DMD and amyotrophic lateral sclerosis (ALS) [8]–[10]. Since it is also sensitive to treatment effects [11], it has the promise of serving as a valuable biomarker in clinical therapeutic trials. EIM relies on the measurement of the voltage generated by the muscle as the response to applying an alternating electrical current, typically between 1 to 1000 kHz. Compared to conventional outcome measures such as the 6MWD or the NSAA, EIM data can be acquired in few seconds per muscle group with little training.

To date, most EIM studies have only relied on single frequency EIM values. For example, the phase or reactance values measured at 50 kHz have been shown to be sensitive to the disease progression in clinical and basic studies. However, modeling the EIM frequency-dependence can help to understand the slope differences where EIM provides reliable sensitivity to the disease progression during the duration of the study. Some of these modeling approaches in the literature include functional principal component analysis (FPCA) based decomposition of signal [12], and local linear regression and weighted least squares estimation techniques including wild and re-sampling bootstrap methods [13]. FPCA and local linear regression approaches are mostly considered data-driven approaches. The estimation procedures in these methods rely on selection of number and type of basis functions, and/or fine-tuning of the parameters. Moreover, they require additional computation to develop the confidence bands around the overall functional fit. An alternate wavelet-based approach with a non-linear shrinkage prior [14] is very general and requires considerable modification for longitudinally measure EIM outcomes. Its estimation also requires Markov chain Monte Carlo (MCMC) computationally intensive algorithm.

To overcome the aforementioned limitations, we propose a novel functional mixed-effects model using state space approach to understand the EIM response trajectories. Our approach imposes a smoothing spline structure on EIM functional response at each visit and taking into account the correlations of these curves along the longitudinal measurements. By recasting the functional mixed-effects models in the state-space form, we achieve optimality of the functional fit in terms of smoothing splines and gain computational efficiency by the employment of the diffuse Kalman filter and smoother (DKFS) algorithm. Furthermore, it allows for several useful generalizations such as the inclusion of exponential decaying patterns and damped oscillatory cycles in the underlying response curve. Finally, the estimates of the posterior variance-covariance matrix of all the hidden states allow for the construction of the component-wise Bayesian confidence bands. These Bayesian confidence bands have been shown to provide a systematic approach for understanding the underlying uncertainty with across-the-function coverage property [15], [16].

Our article is organized as follows: in Section II we describe the materials used in our study. Then, in Section III, we discuss our functional mixed-effects modeling approach for the functional EIM outcome measured longitudinally. We provide the representation of the proposed model in terms of a state-space modeling approach and discuss the ideas behind the estimation of the hidden states and the parameters using a Kalman filter and smoother. In Section IV, we test the performance of our novel model using simulations. Next, our modeling approach is evaluated in Section V from EIM data measured. Finally, the major findings of our work are discussed in Section VI and summarized in the form of conclusion in Section VII. The supplementary material contains the additional plots for individual subjects and PROC SSM SAS code (State-Space Models, SAS version 9.4, Cary, US) along with the explanation of the syntax specification. PROC SSM [17], [18] is a newly developed procedure, which is part of SAS/ETS, that simplifies the specification and estimation of a variety of functional mixed-effects models by using the state-space approach.

II. MATERIALS

A. Electrical impedance myography

The data used to illustrate our proposed methodology is based on a recently completed study assessing EIM as a biomarker in DMD population. The details of the study which took place at Boston Children's Hospital have been described in the articles [8]–[10]. Briefly, the subjects participated in the study for 2 years, undergoing intermittent measurements with EIM as well as physical functioning assessments every few months. The main goal of the study as proposed was to determine how well EIM functioned as potential biomarker to evaluate disease progression and could predict future function. EIM data at 256 frequencies from 1 to 1000 kHz was measured unilaterally in the lateral deltoid, biceps brachii, wrist flexor compartment, rectus femoris, tibialis anterior, and medial gastrocnemius. Data were collected with the ImpSFB7 system (Impedimed, Inc., Brisbane, Australia) placing the electrode probe along the main muscle fibers' direction. Given the complexity of the analysis and our major goal of providing initial proof of principle for this approach, we focused our work only on the deltoid and only on reactance values. If successful, this approach could then be expanded additional muscles, either alone or in combination, as well as the other impedance parameters of resistance and phase.

III. METHODS

In this section, we begin our model formulation using a state space framework. Our model is based on the theory of smoothing splines as it is naturally amenable to state-space representation. The random intercept and slope which define the subject and the subject-time specific deviations are modeled using Gaussian random processes.

A. Model specification

Suppose that the measured EIM response y of a particular muscle (magnitude, phase, resistance, reactance or ratio between values) is the function of frequency $f \in \mathbb{R}^1$ and it is a smooth function in a Sobolev space, *i.e.*, $y \in \mathcal{W}_m^2[0, F]$. Let us further assume that we have $i = 1, \dots, N$ subjects in K groups, and the functional outcomes are measured at $j = 1, \dots, T_i$ time-points. Here, the EIM outcome y were obtained from 1 to 1000 kHz. Let us denote this frequency range as $f \in (0, F)$ with observations obtained at the discrete design points as $f_{ij} = [f_{ij,1}, \dots, f_{ij,n_{ij}}]$. Without any loss of generality, we will assume that $f \in (0, 1)$ where f denotes frequency along which the EIM is sampled. Hence, we can generalize a functional mixed-effects model with longitudinal measurements as follows,

$$y_{ij}(f) = x_{ij}^T \mathcal{B}(f) + z_{ij}^T \mathcal{A}_i(f) + \gamma_{ij}(f) + \varepsilon_{ij}(f), \quad (1)$$

where the vector x_{ij} contains the covariates for the i th subject and j th time-point, and the vector $\mathcal{B}(f) = [\mathcal{B}_1(f), \mathcal{B}_2(f), \dots, \mathcal{B}_P(f)]^T$ contain the $P \times 1$ functional fixed-effects with P a positive integer, *i.e.*, $P \in \mathbb{Z}^+$, and $\top$ denotes the transpose operator. For example, $x_{ij}^T \mathcal{B}(f)$

can be set as $\mathcal{B}_1(f) + \mathcal{B}_2(f)t_{ij} + \mathcal{B}_3(f)t_{ij}^2$ to model the quadratic trend in functional outcomes along the longitudinal measurements, or it can be set as $\mathcal{B}_1(f) + \mathcal{B}_2(f)G + \mathcal{B}_3(f)t_{ij} + \mathcal{B}_4(f)G \times t_{ij}$ to capture the linear change in the functional responses across two groups over the design time-points. The z_{ij} covariates are usually a subset of x_{ij} and the corresponding $Q \times 1$ functional random-effects at the subject level with $Q \in \mathbb{Z}^+$ are denoted by $\mathcal{A}_i(f) = [\mathcal{A}_{i1}(f), \mathcal{A}_{i2}(f), \dots, \mathcal{A}_{iQ}(f)]^\top$ for the i th subject. An additional functional random-intercept at the subject-time level $\gamma_{ij}(f)$ captures heterogeneity in the mean function across various subjects and time-points. The error $\epsilon_{ij}(f)$ are assumed to be zero-mean, square-integrable, mutually uncorrelated random processes as $N(0, \sigma_\epsilon^2)$. Note that the stochastic process $\gamma_{ij}(f)$ which is represented by smooth Gaussian process is assumed to be independent of the noise process $\epsilon_{ij}(f)$. Each functional in the fixed and random-effects vectors can be written in terms of a Gaussian process [15], [19]–[27]. Specifically, the smoothing (polynomial) spline is defined using the integrated Wiener process as,

$$\begin{aligned}\mathcal{B}_p(f) &= \sum_{u=1}^U B_{pu} \frac{f^{u-1}}{(u-1)!} + \lambda_{bp}^{-\frac{1}{2}} \int_0^f \frac{(f-s)^{U-1}}{(U-1)!} \mathcal{W}_p(s) ds, \quad (2) \\ \mathcal{A}_{iq}(f) &= \sum_{v=1}^V A_{iqv} \frac{f^{v-1}}{(v-1)!} + \lambda_{aq}^{-\frac{1}{2}} \int_0^f \frac{(f-s)^{V-1}}{(V-1)!} \mathcal{W}_{iq}(s) ds, \\ \gamma_{ij}(f) &= \sum_{w=1}^W G_{ijw} \frac{f^{w-1}}{(w-1)!} + \lambda_g^{-\frac{1}{2}} \int_0^f \frac{(f-s)^{W-1}}{(W-1)!} \mathcal{W}_{ij}(s) ds\end{aligned}$$

where $\mathcal{W}_p(s)$, $\mathcal{W}_{iq}(s)$ and $\mathcal{W}_{ij}(s)$ are the Wiener processes along with their respective smoothing parameters λ_{bp} , λ_{aq} and λ_g . For the fixed-effects, the parameters B_{pu} are assigned a diffuse prior *i.e.*, $[B_{p1}, B_{p2}, \dots, B_{pU}]^\top \sim N(0, \kappa I)$ with a scalar κ representing the diffuse variance, *i.e.*, $\kappa \rightarrow \infty$ and I is the conformable identity matrix. For the random-effects, the parameters A_{iqv} and G_{ijw} have non-diffuse priors with well defined variance-covariance matrices, *i.e.*, $[A_{iq1}, A_{iq2}, \dots, A_{iqV}]^\top \sim \mathcal{N}(0, \sigma_a^2 D_a)$ and $[G_{ij1}, G_{ij2}, \dots, G_{ijW}]^\top \sim \mathcal{N}(0, \sigma_g^2 D_g)$. We also refer to texts by [28] and [29] for various choice of basis functions and other possible constructions of smoothing splines using the theory of reproducing kernel Hilbert space (RKHS). For our application, we have set $U = V = W = 2$ which represents cubic smoothing spline for the fixed and random-effects.

The functional mixed-effects model in (1) can be represented within a state-space modeling framework. In the next subsection, we present the details of our longitudinal functional mixed-effects model.

B. Model representation in state-space form

For the sake of notational simplicity, we have assumed that the measurements are balanced, *i.e.*, all the subjects have been measured at exactly the same time and the measurements along the frequency are acquired along the same design points, *i.e.*, $T_i = T$ and $f_{ij} = f = [f_1, \dots, f_n]$. Then, the observation and state transition equations for all the measurements of every subject can then be written using a state-space framework at a particular frequency design point f_k identified by index k as

$$\begin{aligned} y_k &= \Phi_k \nu_k + \varepsilon_k, \\ \nu_k &= \Lambda_k \nu_{k-1} + \eta_k, \end{aligned} \quad (3)$$

where the observation vector y_k contains the longitudinal observations of all the subjects stacked as a vector, *i.e.*, $y_k = [y_1(f_k), y_2(f_k), \dots, y_N(f_k)]^\top$. The observation vector of the i -th subject $y_i(f_k)$ in the above representation is defined by stacking the T longitudinal observations $y_i(f_k) = [y_{i1}(f_k), y_{i2}(f_k), \dots, y_{iT}(f_k)]^\top$ at the frequency design point f_k . The state vector contains the functional fixed and random-effects of all the subjects along with their derivatives as $\nu_k = [\mathfrak{B}(f_k), \zeta_1(f_k), \zeta_2(f_k), \dots, \zeta_N(f_k)]^\top$. We refer readers to [30] for the detailed derivation of the state space formulation of functional effects in Equations 2. In this representation, the term $\mathfrak{B}(f_k)$ is defined as the value of functional fixed-effects augmented with (U-1) derivatives, *i.e.*,

$\mathfrak{B}(f_k) = [\mathcal{B}_1(f_k), \mathcal{B}_1^1(f_k), \dots, \mathcal{B}_1^{U-1}(f_k), \dots, \mathcal{B}_P(f_k), \mathcal{B}_P^1(f_k), \dots, \mathcal{B}_P^{U-1}(f_k)]^\top$ at the frequency design point f_k . Therefore, $\mathfrak{B}(f_k)$ has the size $UP \times 1$. Similarly, the state vector $\zeta_i(f_k)$ for the i -th subject contains $VQ \times 1$ functional random-effects at subject level stacked along with $WT \times 1$ functional random-effects at subject-time level and their derivatives, *i.e.*,

$$\begin{aligned} \zeta_i(f_k) &= [\mathcal{A}_{i1}(f_k), \mathcal{A}_{i1}^1(f_k), \dots, \mathcal{A}_{i1}^{V-1}(f_k), \dots, \mathcal{A}_{iQ}(f_k), \mathcal{A}_{iQ}^1(f_k), \dots, \mathcal{A}_{iQ}^{V-1}(f_k), \gamma_{i1}(f_k), \gamma_{i1}^1(f_k), \dots, \gamma_{i1}^{W-1}(f_k), \dots, \gamma_{iT}(f_k), \gamma_{iT}^1(f_k), \dots, \gamma_{iT}^{W-1}(f_k)]^\top \end{aligned}$$

. The observation matrix in the above state space equation 3 for all the N subjects is defined as

$$\Phi_k = \begin{pmatrix} X_1 \Gamma_B & Z_1 \Gamma_\zeta & 0 & 0 & \dots & 0 & 0 \\ X_2 \Gamma_B & 0 & Z_2 \Gamma_\zeta & 0 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ X_N \Gamma_B & 0 & 0 & 0 & \dots & 0 & Z_N \Gamma_\zeta \end{pmatrix},$$

where the corresponding design matrices of the fixed-effects and the random-effects which also includes the random-effect at subject-time level for the i -th subject at the design-points f_k in the observation equation are restructured as

$$X_i = \begin{pmatrix} x_{i1} \\ x_{i2} \\ \vdots \\ x_{iT} \end{pmatrix}_{T \times P} \quad \text{and} \quad Z_i = \begin{pmatrix} z_{i1} & 1 & 0 & \cdots & 0 \\ z_{i2} & 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ z_{iT} & 0 & 0 & \cdots & 1 \end{pmatrix}_{T \times (Q+T)}.$$

The matrix Γ_B of size $P \times UP$ relates the observation vector to the fixed-effects state vector. Since there are no observations pertaining to the derivatives in the outcome vector, it contains 1s and 0s for extracting the respective functional fixed-effects and leaving out the $(U-1)$ derivatives. For example, with $U=2$, the Γ_B is designed to remove the first order derivative, *i.e.*,

$$\Gamma_B = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & \cdots & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 1 & 0 \end{pmatrix}_{P \times 2P}, \quad (4)$$

Similarly, the matrix Γ_ζ of size $(Q+T) \times (VQ+WT)$ is designed using 1s and 0s for extracting the functional random-effects and leaving out the derivatives.

The state transition matrix for the entire state is defined by the block-diagonal stacking of state vector transition matrices corresponding to fixed and random-effects, *i.e.*,

$\Lambda_k = \text{Block-Diag} \left[T_B(f_k), T_{\zeta_1}(f_k), T_{\zeta_2}(f_k), \dots, T_{\zeta_N}(f_k) \right]$, with the state transition matrices of the fixed and random-effects defined in terms of underlying smoothing structure $H_{U f_k}$, $H_{V f_k}$

and $H_{W f_k}$, *i.e.*, $T_B(f_k) = H_{U f_k} \otimes I_P$, and $T_{\zeta_i}(f_k) = \text{Block-Diag} \left[H_{V f_k} \otimes I_Q, H_{W f_k} \otimes I_T \right]$,

where the operator $\otimes$ denotes the Kronecker product. The matrix $H_{U f_k}$ for the smoothing spline is defined as

$$H_{f_k} = \begin{pmatrix} 1 & (f_k - f_{k-1}) & \frac{(f_k - f_{k-1})^2}{2!} & \cdots & \frac{(f_k - f_{k-1})^{U-1}}{(U-1)!} \\ 0 & 1 & (f_k - f_{k-1}) & \cdots & \frac{(f_k - f_{k-1})^{U-2}}{(U-2)!} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \vdots & \vdots & \vdots & \ddots & (f_k - f_{k-1}) \\ 0 & 0 & 0 & \cdots & 1 \end{pmatrix},$$

with ! denoting the factorial operation. The matrices $H_{V f_k}$ and $H_{W f_k}$ are similarly defined in terms of degrees V and W .

The errors in the observation equation of the state space model, *i.e.*, ε_k , are assumed to be independent and identically normally distributed, *i.e.*, $\mathcal{N}(0, \sigma_\varepsilon^2 I)$. The errors in the state equation, *i.e.*, η_k , are defined by the multivariate normally distributed with mean 0 and variance-covariance matrix defined by the Gaussian processes in equation 2. In particular, the variance-covariance matrix of the entire state can be first written as the block diagonal matrices of the variance-covariance matrices pertaining to the state vectors of fixed and random-effects,

$$\sum_{\eta} (f_k) = \text{Block-Diag} \left[\Sigma_B (f_k), \Sigma_{\zeta_1} (f_k), \Sigma_{\zeta_2} (f_k), \dots, \Sigma_{\zeta_N} (f_k) \right],$$

with the state disturbance variance-covariance matrix corresponding to the fixed-effects defined in terms of the respective smoothing parameters and underlying Gaussian process for the smoothing spline, *i.e.*, $\Sigma_B (f_k) = \text{Block-Diag} \left[\lambda_{b1}^{-1} \Sigma_{U f_k}, \lambda_{b2}^{-1} \Sigma_{U f_k}, \dots, \lambda_{bP}^{-1} \Sigma_{U f_k} \right]$.

Similarly, the variance-covariance matrix of the random-effects is defined as

$\Sigma_{\zeta_i} (f_k) = \text{Block-Diag} \left[\lambda_{a1}^{-1} \Sigma_{V f_k}, \dots, \lambda_{aQ}^{-1} \Sigma_{V f_k}, \lambda_{g1}^{-1} \Sigma_{W f_k}, \dots, \lambda_{gT}^{-1} \Sigma_{W f_k} \right]$. The smoothing parameters corresponding to the random-effects at subject-time level are all set equal in our model as defined in equation 2, *i.e.*, $\lambda_{g1} = \lambda_{g2} = \dots = \lambda_{gT}$. The (r, s) -th element of the variance-covariance matrix $\Sigma_{U f_k}$ is given by

$$\sum_{f_k} (r, s) = \frac{(f_k - f_{k-1})^{2U - r - s + 1}}{(2U - r - s + 1)!(U - r + 1)!(U - s + 1)!}.$$

Again, the matrices $\Sigma_{V f_k}$ and $\Sigma_{W f_k}$ are defined in terms of V and W .

To complete the representation of the model in state-space form, the variance-covariance matrices for the initial conditions of the states corresponding to the fixed and random-effects are denoted by the $\Sigma_B(0)$ and $\Sigma_{\zeta_i}(0)$. Specifically, the initial conditions for the states that

correspond to the fixed-effects, are assumed to be diffuse, *i.e.*, it is specified as normally distributed with mean 0 and variance-covariance matrix, $\Sigma_B(0) = \kappa I_U \otimes I_P$ with a scalar $\kappa \rightarrow \infty$, and I_U and I_P are identity matrices of size $U \times U$ and $P \times P$, respectively. On the other hand, the initial conditions for the states that correspond to the random-effects are assumed to be non-diffuse. They are specified as normally distributed with mean 0 and variance-covariance matrix $\sum_{\zeta_i}(0) = \text{Block-Diag} \left[\sigma_{a1}^2 D_a, \dots, \sigma_{aQ}^2 D_a, \sigma_{g1}^2 D_g, \dots, \sigma_{gT}^2 D_g \right]$ with

$\sigma_{g1}^2 = \dots = \sigma_{gT}^2$. In this representation, the random-effects variance-covariance matrix parameters in the null (parametric) space for the subject level is denoted by $\sigma_a^2 D_a$ and for the subject-time level is denoted by $\sigma_g^2 D_g$.

At this moment, the model has been formulated in a state-space form and so the well-known Kalman filter and smoother algorithm can be used for model fitting and functional effects estimation. The next section briefly describes the details of these computations.

C. Model fitting and functional effect estimation

The Kalman filter and smoother (KFS) algorithm is the main computational tool for using state-space models (SSMs) for data analysis. For proper treatment of SSMs with a diffuse initial condition, a modified version of the traditional KFS called diffuse Kalman filter and smoother (DKFS), is needed. A good discussion of the different variants of the traditional and diffuse KFS can be found in [31].

For the current model in equation 3, the model parameters in the state space specification correspond to the different smoothing parameters and the variances of different error terms, *i.e.*, $\theta = (\lambda_{bp}, \lambda_{aq}, \lambda_g, \sigma_{aq}, \sigma_g, \sigma_e)$ with $p = 1, \dots, P$ and $q = 1, \dots, Q$. All of these parameters are estimated by maximizing the diffuse likelihood of the data, which is computed by using the filtering part of the DKFS algorithm. The diffuse likelihood is also known as the restricted likelihood and the estimates obtained by maximizing this likelihood are known as REML estimates. The maximization of the likelihood can be performed by using standard gradient descent based methods (e.g., Newton-Raphson). Once the model parameters are estimated, the functional effects are estimated by using the smoothing part of the DKFS algorithm. Note that the functional fixed and random-effects are linear combinations of the hidden states in the state-space form of the model in equation 3. The smoothing part of the DKFS algorithm yields the estimates of all hidden states (and the standard errors of these state estimates), which in turn provides the estimates of the functional effects (and their standard errors). Finally, the precision estimates of the functional effects are used to construct component-wise Bayesian confidence bands [15], [16], which provide a systematic approach to understanding the underlying uncertainty with the “across-the-function” coverage property.

An important advantage of recasting the functional mixed-effects models in the state-space form is the computational efficiency of the DKFS algorithm; the computational cost of a filtering/smoothing pass is linear in terms of the total number of measurements $\mathcal{O}(\mathcal{N} \approx N \times T)$. Additionally, the state-space form permits many useful generalizations of the traditional functional mixed-effects models that can incorporate more general functional effects such as damped cycles, exponentials, decay and other patterns; for more information see [18]. The formulation in [18] is based on an alternate connection between the stochastic differential equation representation of the functional mixed-effects models and its discrete time model formulation in terms of the design matrices $T_B(f_k)$ and $T_{\zeta_i}(f_k)$ and the state disturbance covariance matrices $\Sigma_B(f_k)$ and $\Sigma_{\zeta_i}(f_k)$. The restriction of unequal

measurements and missing data in state-space form can easily be handled by careful specification of index variables in the notation and letting the state equation iterate one step forward without any modification to the hidden states.

Note 1: If we set $\lambda_{bp} \rightarrow 0$, $\lambda_{aq} \rightarrow 0$ and $\lambda_g \rightarrow 0$, then the functional model representation in the model (1) approaches to the set of independent linear mixed-effects (LME) models for longitudinal measurements at each design point. The integrated Wiener process specification in (2) imposes a cubic smoothing spline structure on the functional outcome.

Note 2: The model in equation 1 can also be represented as a single LME model which can be estimated using a standard software for mixed models (*e.g.*, PROC MIXED in SAS or lme4 in R software). The restricted maximum likelihood (REML) approach, which is commonly used to fit the LME models, often outperforms the generalized cross-validation approach that is popular in functional mixed-effects modeling [24], [25]. However, the estimation based on LME formulation for our model is computationally and memory-wise highly inefficient because it requires creation, storage, and inversion of large matrices that may not be sparse (see, for example, [18]). The computational inefficiency of their SAS MACRO Fmixed implemented by the LME formulation has been discussed in [32].

IV. SIMULATION RESULTS

In this section, we study the coverage properties of the Bayesian confidence bands for the slope parameter using a model which is similar to our motivating example presented in Section 2. The 95% Bayesian confidence band has “across-the-function” coverage property implying that 95% of the curve showing the significance is contained in the band. In our simulation study, we have generated data using the following model for $i = 1, \dots, N$ and $j = 1, \dots, T$ as

$$y_{ij}(f) = \mathcal{B}_1(f) + \mathcal{B}_2(f)G + \mathcal{B}_3(f)t_{ij} + \mathcal{B}_4(f)G \times t_{ij} \quad (5) \\ + \mathcal{A}_{1i}(f) + \mathcal{A}_{2i}(f)t_{ij} + \gamma_{ij}(f) + \varepsilon_{ij}(f),$$

The functions for the fixed-effects are set as $\mathcal{B}_1(f) = 1/(1.25 + 0.5(1.5 - f)^3)$, $\mathcal{B}_2(f) = 0.006 - 0.001\sqrt{(f + 0.075)}$, $\mathcal{B}_3(f) = 1/(2 + 0.02(1.1 - f)^2)$ and $\mathcal{B}_4(f) = -0.05 + 0.002e^{3(1.05 - f)}$ with $f \in (0, 1)$. For the simulations to be realistic, the error and random-effects variance parameters in the simulations were set close to the estimates obtained in our EIM data. The data were scaled by a factor of 100 prior to model fitting to prevent rounding floating point errors in the recursive DKFS algorithm.

The Bayesian 95% “across-the-function” coverage property for the main parameter $\mathcal{B}_4(f)$ is computed for $N = 25, 30, 35$ and 40 subjects. The study time was set uniformly as $t = 1, 2, \dots, T$ with $T = 3, 5$ and 7 time-points. Similarly, the frequency range was set on a uniform grid of $(0, 1)$ with 1000 sampling points. Table I shows that the median of 95% coverage is close to the desired level even for the modest number of time-points and subjects. The other fixed-effects functionals in the model 5, *i.e.*, $\mathcal{B}_1(f)$, $\mathcal{B}_2(f)$ and $\mathcal{B}_3(f)$, showed similar performance in terms of the coverage property and these simulation findings are consistent with the earlier reported results by [15] and [16].

V. EXPERIMENTAL RESULTS

We evaluated phase, resistance and reactance and considered the reactance only measured along the muscle major axis for the deltoid muscle of the subjects. In order to normalize the scale (dimensionless) and stabilize the model optimization, the deltoid reactance ratio (DRR) was defined as the ratio of reactance measured at individual frequency sampling point and dividing it by the reactance value measured at 300 kHz. In addition, the DRR was scaled by a factor of 100 also to numerically stabilize the model optimization. In this cohort, we have 16 boys with DMD whose mean age is 10.16 (SD 2.0) years and 12 healthy controls with a mean age of 9.86 (SD 2.4) years. The longitudinal observations were obtained at baseline, 5 days, 1, 2, 3, 6, 9, 12, 18 and 24 months.

Our primary model to estimate DRR longitudinal differences between the two populations is as follows

$$y_{ij}(f) = \mathcal{B}_1(f) + \mathcal{B}_2(f)G + \mathcal{B}_3(f)t_{ij} + \mathcal{B}_4(f)G \times t_{ij} \quad (6) \\ + \mathcal{B}_5(f)\text{Age} + \mathcal{A}_{1i}(f) + \mathcal{A}_{2i}(f)t_{ij} + \gamma_{ij}(f) \\ + \varepsilon_{ij}(f).$$

We compared the model in equation 6 with several nested models listed in Table II. Table II summarizes the findings in terms of Akaike information criterion (AIC) for these nested models. The AIC for the model with the random intercept at the subject level is obtained as 78094.29; whereas it is obtained as 22204.26 when the random intercept is included at the subject-time level instead. Hence, the dynamic random-effect at the subject-time level plays a stronger role in the final model fit in comparison to random-effects at the subject level. The AIC of Model 6 in equation 6 which includes all the random-effects is a little bit larger than Model 5 with only random intercepts at the subject and the subject-time levels (Model 6 AIC = 22318.82 vs Model 5 AIC = 22101.55). This finding implies that inclusion of the random-effect at the slope level might lead to a little bit of over-fitting for our current data. Lastly, we also allowed the log of error variance to be a function of the B-spline basis function of degree 2 with the specification $(\sigma_e^2) = \sum_{j=1}^{d+r+1} c_j \Psi_{jd}$, where Ψ_{jd} for $j = 1, \dots, (d+r+1)$ are the B-spline basis functions with r knots of degree d and order $(d+1)$. The coefficients of B-spline basis functions are optimized along with the other parameters, *i.e.*, $\theta = (\lambda_{bp}, \lambda_{aq}, \lambda_g, \sigma_{aq}, \sigma_g, \sigma_e, c_j)$.

Figures 1(a) and 1(b) provide the observed plots of DRR for the boys for the frequency range of 30–100 kHz. Although the two surface shapes are clearly distinct and there is a significant difference in the entire frequency range of the baseline curves; it is hard to discern the longitudinal differences between the two populations from the original measured response. The plots of the age-adjusted mean response of control and DMD are shown in Figures 1(c) and 1(d). Figures 1(e) and 1(f) show the smooth estimate of the DRR mean response based on the model 6.

The plots of estimated slopes for the two-populations are shown in Figures 2(a)–2(b). Figures 2(a) and 2(b) show that the slopes of both Control and DMD population curves start

decreasing in a curvilinear fashion with higher frequencies. The plot of estimated slope difference (main parameter of interest $\mathcal{B}_4(f)$) along with its Bayesian 95% confidence band are shown in Figures 2(c) and 2(d) for the Models 6 and 7. Figure 2(c) shows that the slope difference between the two populations starts increasing almost linearly with higher frequencies. However, it is clear from Figure 2(d) that this effect is not significant beyond 70 kHz for our current sample size in this age group. Bayesian 95% confidence bands generated using the Model 7 are a little narrower than the Model 6 for the frequency range of 30–70 kHz as they account for the changing error variance (Figure 2(f) of the DRR measure along the frequency. Figure 2(e) shows the slope difference estimated using the Model 7 without the corresponding 95% confidence band. The DKFS filtering and smoothing algorithm yield the posterior estimates of all hidden states along with posterior variance-covariance matrix which can be used to construct the Bayesian confidence bands. Note that the 95% Bayesian confidence upper and lower limits of the slope difference are curvilinear in our data. The functional slope difference is not easily visible without an overall single model and hence the identification of the frequency range is very difficult for various muscle groups and other EIM derived measures which can be quite large in EIM based studies.

Additionally, Figure 3 shows the baseline age effect estimated from the model. The component-wise Bayesian 95% confidence band constructed using the posterior variance-covariance estimates show the effect starts getting close to losing significance for higher frequencies. We further note that the estimated baseline age effect Bayesian 95% upper and lower confidence limits are systematic along the frequency, although the band shows large uncertainty around it due to small sample size.

The supporting material contains the additional plots and the details of PROC SSM.

VI. DISCUSSION

For a comprehensive review and detailed reading on smoothing splines, we also refer to texts [28] and [29]. In the literature, the smoothing spline modeling approach for multiple subject functional outcomes have been extended by inclusion of a well-defined zero-mean stochastic processes for the random-effects [33]–[35]. These modeling approaches capture features such as tensor product specification in the two-dimensional setting or incorporation of autoregressive and feedback relationships between bi-variate functional outcomes. The estimation of the functionals in these models have been based on the signal extraction approach [30] relying on the state space formulation. The functional mixed-effects model in [33] and [35] were defined to model the data with functional responses measured only on one occasion for each subject. The approach proposed by [34] did not include random-effects. Our approach also differs from other approaches which are either sub-optimal such as penalized spline or based on data drive orthonormal basis function expansions, see for example [36]–[38].

In this article, we have extended the functional mixed-effects model to model the responses measured at multiple visits while accounting for between-subject heterogeneity and within-subject correlations along the longitudinal direction. The smoothing spline approach naturally extends modeling of functional outcomes measured at several occasions contrary to

the claims in the previous articles [12], [13]. Furthermore, the state-space formulation allows for estimation of the functional fixed and random-effects linear in time. In this article, we have not evaluated the performance of smoothing spline formulation with the FPCA and local linear regression based approaches. Further work is needed to compare these approaches in terms of ease of implementation and performance of simultaneous confidence intervals for the functional fixed-effects. Although we have presented our model using cubic smoothing spline, the state-space model presented by [18], [33], [35] for the functional mixed-effects model can accommodate many kinds of smoothing spline structures. The number of estimated parameters using an independent set of LME models for EIM measure at each frequency increases drastically with the sampling rate. Moreover, the estimates obtained using these independent set of LME models provide an incomplete picture or sometimes contradictory results for each muscle group. Our proposed approach provides a unified framework to test the differences in functionals longitudinally which makes it statistical efficient and robust to random fluctuations. The smoothing splines can also allow for different bandwidths very similar to wavelets based approaches by the modification of the ratio of variance components (smoothing parameter and variance of the residual). This approach can be extended to jointly model the multiple EIM features acquired from several muscle groups by imposing correlations among the null space parameters of the functional random-effects. However, this complex problem would require high performance computing. Finally, our modeling approach can be easily applied in other fields such as neuromuscular degenerative disease areas and neuroimaging diffusion tensor imaging fiber tracts studies.

VII. CONCLUSIONS

In this manuscript, we have proposed an innovative approach which simultaneously models the functional response while incorporating random-effects at subject level and subject-time level and hence thereby, capturing the correlation structure along the frequency and longitudinal directions. Our modeling approach allows us to differentiate the smooth response of the underlying signal longitudinally without any parametric restriction and allows for separation of the subject-specific deviations from the population-averaged response surface. We envision that this new tool could be used in clinical studies to validate the functional biomarkers. We have included easy-to-implement code for the practitioners in the supplementary section.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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(a) Control

(b) DMD

(c) Control

(d) DMD

(e) Control

(f) DMD

Fig. 1. Deltoid reactance ratio (DRR) (a-b) Control and Duchenne muscular dystrophy (DMD) observed mean (c-d) Control and DMD age adjusted mean (e-f) Control and DMD model fit.

(a) Control Slope estimate

(b) DMD Slope estimate

(c) Slope difference Model 6

(d) Slope difference Model 7

Fig. 2. Smooth estimates of a) Control slope b) Duchenne muscular dystrophy (DMD) slope c) Slope difference d) Error variance function estimate (e-f) Slope difference along with Bayesian 95% confidence band of Model 6 and Model 7.

Fig. 3. Smoothed estimate baseline age effect $\mathcal{B}_5(f)$ along with the component-wise Bayesian 95% confidence band.

TABLE I

95% COVERAGE RESULTS IN TERMS OF MEDIAN (INTER-QUARTILE RANGE)

N	T = 3	T = 5	T = 7
25	93.7 (66.1–99.7)	92.1 (68.5–97.2)	93.1 (70.2–97.5)
30	94.8 (70.5–98.2)	94.8 (72.2–96.4)	94.7 (73.6–95.8)
35	96.9 (69.4–97.3)	96.1 (70.6–98.4)	95.3 (72.1–96.2)
40	95.5 (69.2–97.1)	95.8 (72.3–97.1)	95.2 (71.6–97.3)

TABLE II

AKAIKE INFORMATION CRITERION (AIC) FOR VARIOUS NESTED MODELS

Model	Detail	AIC
Model 1	Only fixed-effects	90399.54
Model 2	Only random intercept at subject level	78094.29
Model 3	Only random intercept and slope at subject level	75511.78
Model 4	Only random intercept at subject-time level	22204.26
Model 5	Same as model 4 with random intercept at subject level	22101.55
Model 6	All the random-effects (model 6)	22318.82
Model 7	Same as model 6 along with variance function	21708.15