Advanced Methodological Framework for Interpretable Machine Learning in Autism Spectrum Disorder using Heterogeneous Resting-State fMRI Data

I. The ABIDE Heterogeneity Crisis: Mandatory Data Harmonization

The challenges encountered when developing robust Machine Learning (ML) models on large, multi-site neuroimaging repositories such as the Autism Brain Imaging Data Exchange (ABIDE) are fundamentally rooted in uncontrolled data variability. This site-specific noise directly obscures the subtle functional connectivity (FC) biomarkers associated with Autism Spectrum Disorder (ASD), leading to model opacity and poor generalizability.

A. The Statistical Impact of Multi-Site Variability

The ABIDE consortium, while crucial for advancing large-scale neuroimaging research, presents inherent limitations due to its aggregated nature. The collected datasets are afflicted by non-pathological variability, often referred to as "batch effects".[1] These variations arise from fundamental differences across acquisition centers, including scanner magnetic strength, vendor disparities, inconsistencies in MR protocols, magnetic homogeneity, and variations in reconstruction algorithms.[1]

When ML models are trained on raw, unharmonized multi-site data, these disparities limit the power required to detect genuine neurobiological differences between clinical and typically developed (TD) groups. Crucially, the classifier tends to learn characteristics of the acquisition site—the batch effects—rather than the underlying biological pathology. This phenomenon results in models that perform poorly in cross-site validation (e.g.,

leave-one-site-out strategies) and contributes directly to the unexplainable nature of their predictions, as the features driving classification are artifactual noise rather than true neural signals.[1]

B. Prescriptive Solution: ComBat Harmonization

To ensure that ML classifiers prioritize biological variance, robust statistical harmonization of the feature space is a mandatory prerequisite. The **Combating Batch (ComBat)** technique, originally developed for genomic studies, has been successfully adapted to neuroimaging data, providing a robust solution for standardizing heterogeneous FC measures.

The mechanism of ComBat involves adjusting the feature data by modeling site-related means and variances as empirical priors, effectively removing the nuisance variance associated with site acquisition while preserving the underlying biological signal.[3] This approach ensures that the statistical relationship between the connectivity features and biological covariates, such as age or clinical metrics, is maintained or even enhanced after site effects are eliminated.[4]

Empirical validation in connectomics affirms the necessity of this approach. Studies have demonstrated that ComBat successfully removes site effects identified in FC and network measures. This harmonization step simultaneously increases the statistical power available to detect subtle biological associations, even across various connectivity metrics and brain atlases.[3] Furthermore, the impact on classification performance is significant and dramatic. Studies utilizing multi-site data show that harmonization elevates accuracy substantially. For instance, in related radiomics research, classification accuracy improved dramatically from a range of 34%–75% before harmonization to 89%–96% after ComBat application.[6] Applied specifically to ABIDE data, ComBat harmonization achieved higher classification accuracies across multiple models, particularly those based on artificial neural networks, outperforming existing results from unharmonized data.[1]

The essential understanding here is that harmonization is the foundational requirement for model interpretability. If an ML model's decision function is primarily based on arbitrary scanner noise (batch effects), any subsequent Explainable AI (XAI) analysis will falsely attribute importance to non-biological features. Therefore, ComBat purification of the input space is a critical preprocessing step that guarantees XAI targets the variance associated with genuine neural alterations in ASD.

It is necessary to approach ComBat application with quantitative rigor, as the magnitude of site effects is not constant across all extracted features. The necessity of rigorous validation stems from the observation that site variability is non-uniform and depends heavily on the specific connectivity metrics employed (e.g., Pearson correlation versus wavelet coherence)

and the chosen parcellation scheme.[5] Therefore, a researcher must quantitatively assess and confirm the effectiveness of ComBat adjustment on their specific set of high-dimensional features (e.g., graph theory metrics or dynamic connectivity parameters) before proceeding to model training.

C. Alternative Harmonization Approaches

While ComBat is highly effective for post-feature extraction harmonization, alternative methods exist, particularly when the goal is to fully leverage the $4D$ spatial-temporal structure of the fMRI data. Domain adaptation techniques, such as adversarial learning or low-rank representation, have been proposed for multi-site ASD classification.[7] These methods aim to transform data into a common space, minimizing heterogeneity between sites while preserving both the spatial and temporal information of the original resting-state fMRI (rs-fMRI) data, leading to superior performance gains.[7]

The following table summarizes the observed empirical benefits of data harmonization using the ComBat technique across neuroimaging studies:

Table Title: Comparative Impact of ComBat Harmonization on Classification Performance

Study Focus	Input Data Type	Pre-ComBat Performance	Post-ComBat Performance	Key Outcome
PD Classification	Radiomic Features	34%–75% Accuracy	89%–96% Accuracy	Dramatic improvement in classification accuracy [6]
Age Association	FC & Network Measures	Site effects significant	Increased statistical power to detect associations	Site effects successfully removed, biological factors preserved [3]
ASD Classification	Connectomes	Outperformed	Higher classification	Confirms efficacy for

(Multi-site)	(ANN)	existing results	accuracies achieved	ABIDE data using neural networks [1]

II. Resolving r-fMRI Ambiguity: Preprocessing for Feature Stability

The interpretability and predictive stability of ML models are inextricably linked to the upstream quality control and preprocessing steps applied to the rs-fMRI time series. Two highly consequential decisions involve motion correction and the implementation of Global Signal Regression (GSR).

A. Motion Correction: Interpolation vs. Scrubbing

Head motion introduces structured artifacts into fMRI time series that can masquerade as changes in functional connectivity, particularly in clinical populations.[1] Rigorous mitigation of these effects is crucial. While "scrubbing" (removing volumes that exceed a motion threshold) is a common strategy, it reduces the effective length of the time series, undermining statistical power and potentially violating the continuity assumptions required by temporal analysis methods.

Research suggests that combining a parsimonious model using 6 Motion Parameters (MPs) with **volume interpolation** of motion outliers provides a favorable methodological compromise, often surpassing the performance of scrubbing methods.[9] Volume interpolation is an easy-to-implement technique that corrects artifactual signal spikes while preserving the temporal continuity and length of the fMRI data.[9] This preservation of temporal continuity is essential for the stability and statistical validity of Dynamic Functional Connectivity (dFC) analysis techniques, such as sliding window approaches, which rely on having a continuous sequence of data points.

B. The Global Signal Regression (GSR) Debate in ML Context

GSR involves removing the mean signal averaged across the entire brain, a step considered controversial in the neuroimaging community.[11] On one hand, the global signal contains substantial variance attributable to physiological confounds such as cardiac and breathing fluctuations, as well as head motion.[11] Regressing this signal effectively reduces artifactual connectivity and nuisance variance.[11] On the other hand, a component of the global signal may reflect true, widespread neural activity, and its removal mathematically imposes anti-correlations, potentially distorting true brain network relationships.[12]

However, neurophysiological evidence supports the utility of GSR in a performance-driven context. Simultaneous electroencephalography (EEG)-fMRI studies demonstrate that systemic physiological fluctuations account for a significantly larger fraction of global signal variability than electrophysiological fluctuations. Crucially, GSR was shown to reduce artifactual connectivity caused by heart rate and breathing variations while *preserving* connectivity patterns linked to electrophysiological activity within the alpha and beta frequency ranges.[11] This evidence suggests that the neural component of resting-state FC is largely retained after global signal removal.

For classification tasks, the inclusion of GSR often translates to empirical performance gains. Studies using ML to classify ASD on the ABIDE dataset have shown that models trained on features derived from correlation matrices that include anti-correlations (a common outcome of GSR) lead to higher classification accuracy and Area Under the Curve (AUC) scores.[14] While the biological origin of GSR-induced anti-correlations is complex, their statistical informativeness in distinguishing ASD from controls is robust.[15]

Therefore, a prescribed approach for maximizing ML efficacy involves empirically testing preprocessing pipelines both with and without GSR. Specifically, researchers should compare the performance of the filt_noglobal strategy (standard nuisance regression) against the filt_global strategy (including GSR).[16] If the latter yields significantly higher classification performance following data harmonization, its selection for diagnostic purposes is justified, although interpretations of specific anti-correlations must remain cautious and contextualized.

III. Advanced Feature Engineering: Leveraging 4D Data and Optimized Atlases

The inherent ambiguity encountered in r-fMRI analysis often stems from inadequate feature representation. Moving beyond simple, static summaries and optimizing the spatial definition of brain regions are critical steps in maximizing predictive power and interpretability.

A. The Shift from Static (3D) to Dynamic (4D) Functional Connectivity

Traditional Static Functional Connectivity (SFC) calculates a single correlation matrix (a $3D$ representation) over the entire duration of the fMRI scan. This approach, by averaging temporal information, inherently limits the model's ability to capture the dynamic and time-varying nature of neural organization.[17] Since neurological disorders, including ASD, manifest subtle, temporal-shifting network irregularities, reliance on SFC results in a significant loss of crucial information.[1]

Dynamic Functional Connectivity (dFC) overcomes this limitation by treating the fMRI data as a $4D$ spatial-temporal signal. Utilizing sliding window techniques, dFC captures the rapid reconfigurations and temporal dependencies of brain network states.[7] Empirical evidence strongly supports this methodological shift, demonstrating that dFC-based classifiers yield superior classification power, enhanced stability, and higher accuracy compared to their SFC counterparts.[17] One study reported a classifier accuracy of 100% using dFC features, contrasting sharply with only 50% accuracy achieved by a static classifier on the same random atlas features.[19]

To effectively model dFC features, which involve sequences of connectivity matrices, specialized Deep Learning architectures are necessary. Recurrent Neural Networks (RNNs) or, more specifically, Long Short-Term Memory (LSTM) networks are recommended for modeling the temporal dependencies in brain functional connectivity.[20] These models can explicitly learn the "atypical temporal dependencies" found in the ASD connectome, potentially serving as reliable biomarkers.[20] Given the subtle and heterogeneous nature of ASD, treating the data as a time series of connectivity states rather than a singular static map is an imperative step towards extracting maximum discriminative features. Optimal performance is often realized through the combined use of static and dynamic features, recognizing that both the mean strength (SFC) and the temporal variability (dFC) contribute unique biological information relevant for classification.[17]

B. The Impact of Brain Atlas Selection

The choice of brain parcellation scheme, or atlas, fundamentally determines the quality and meaning of extracted FC features. The atlas defines the Regions of Interest (ROIs), dictating the feature dimensionality, computational load, and biological specificity of the resulting

connectome.[22]

For detecting subtle pathologies like ASD, where neural differences are nuanced, dense and functionally derived atlases are generally superior to coarse, purely anatomical parcellations (e.g., AAL). Denser atlases, such as CC400, Schaefer (with hundreds of nodes), or Glasser 360, provide higher spatial granularity.[22] Functional parcellations, which define regions based on intrinsic connectivity homogeneity or multi-modal cortical architecture [25], yield more biologically robust ROIs for studying network dynamics.

Studies investigating ASD classification confirm that atlas selection significantly affects classification accuracy.[22] While coarser atlases offer computational efficiency, higher granularity is typically necessary for superior feature definition. Furthermore, specific atlas and feature combinations have been empirically proven to be highly discriminative, such as the combination of the MODL atlas with tangent features, achieving high classification success.[26] Therefore, empirical testing across multiple state-of-the-art functional atlases is essential for optimizing the feature space for ASD classification.

Table Title: Atlas Granularity and Feature Selection Trade-offs in Classification

Atlas Type/Example	**Granularity**	**Node Count (Example)**	**Primary Advantage**	**Classification Implication**
Coarse Anatomical (AAL)	Low	90–116	Computational Efficiency	Risk of masking differences by averaging functionally distinct signals [22]
Functional (Schaefer, Glasser)	High	360–400+	Higher Biological Specificity	Enables detailed network definition and potentially higher accuracy [24]
Discriminative (MODL)	Varies	Varies	Empirically optimized FC features	Proven effectiveness when paired

				with specialized feature transformation [26]

IV. Establishing Normative Baselines: Defining the "Normal Threshold"

The mandate to define a "normal threshold" for ASD classification requires transitioning from traditional binary group classification to individualized, quantitative assessment using Normative Modeling. This framework provides the statistical rigor necessary to define a healthy range against which individual deviations can be measured.

A. Statistical Definition of a Multivariate Normal Baseline

Functional connectivity analysis generates a high-dimensional feature vector comprised of $N(N-1)/2$ connections for an N-node atlas. The statistical concept of a "normal threshold" for this vector must be defined within the context of the **Multivariate Normal Distribution (MVD)**, which generalizes the univariate normal distribution to higher dimensions.[27] A random vector is considered MVD if every linear combination of its components has a univariate normal distribution.[27]

The reliance on the MVD is justified by the Multivariate Central Limit Theorem, which ensures that the sample mean vector extracted from large, independent, and identically distributed samples (such as a large TD cohort in ABIDE) will be approximately multivariate normally distributed.[28] This provides the mathematical foundation necessary for robust statistical inference in high dimensions.

To operationalize the "normal threshold," the mean vector $\boldsymbol{\mu}$ and the covariance matrix $\boldsymbol{\Sigma}$ of the TD population's FC feature space are estimated. The Mahalanobis distance, D_M, can then be calculated for any individual subject's FC vector $\mathbf{x}$, quantifying its distance from the mean of the healthy population:

$$D_M(\mathbf{x}) = \sqrt{(\mathbf{x} - \boldsymbol{\mu})^T \boldsymbol{\Sigma}^{-1} (\mathbf{x} - \boldsymbol{\mu})}$$
Subjects whose Mahalanobis distance exceeds a pre-defined critical threshold (typically derived from a chi-squared distribution, given the dimensionality) are classified as multivariate statistical outliers, providing a statistically rigorous definition of abnormality relative to the healthy population.

B. The Normative Modeling Framework

Normative modeling represents the most advanced approach to individualized neurological assessment. This framework involves building sophisticated statistical models that predict the expected FC profile (or other brain features) of a neurotypical individual across continuous covariates like age, sex, and acquisition site.

The core utility of normative modeling lies in its output: the **Deviation Z-score**. This metric quantifies the degree and direction of abnormality (hypo- or hyper-connectivity) for every individual FC feature relative to the established healthy expectation.[29] These personalized deviation maps are vital for disentangling the profound heterogeneity characteristic of ASD. Studies have shown that applying this framework allows for the decomposition of ASD into functionally distinct subtypes, revealing idiosyncratic functional network topologies associated with specific behavioral impairments (e.g., perception, language, or socio-emotional functioning).[29]

Crucially, the researcher should employ a methodological shift: instead of using the raw FC matrices as input features for ML, the deviation Z-scores derived from the normative model should serve as the primary feature space. This reframes the classification task from detecting subtle, heterogeneous group differences to identifying common patterns of *individualized brain abnormality*. By using Z-scores, the model directly optimizes on clinically relevant variance, potentially leading to more accurate, generalizable, and clinically meaningful outcomes.

V. Building Interpretable ML Models (Explainable AI/XAI)

The central requirement for research success is not merely high prediction accuracy but the ability to translate ML decisions back into verifiable neurobiological features. This necessity

demands the rigorous application of Explainable AI (XAI) methodologies to transcend the "black box" nature of complex classifiers.

A. XAI Methodologies for Dynamic Functional Data

The primary goal of XAI in this context is to assign relevance scores to specific input features (individual functional connections, time points, or derived network metrics) that contribute most strongly to the model's diagnostic classification.[31]

For deep learning architectures, particularly those used to analyze $4D$ fMRI data like RNNs or LSTMs, gradient-based attribution methods are essential tools. Techniques such as **Integrated Gradients** and **DeepLift** have been systematically validated for functional neuroimaging analysis.[21] These methods successfully identify ground-truth affected regions even under challenging conditions, including low signal-to-noise ratio ($–10$ dB SNR), low prevalence of affected regions, and subtle pathological alterations.[21] This validation establishes the reliability of these XAI methods for functional connectome analysis.

Feature selection can also be embedded directly into the ML pipeline to enhance interpretability:

1. **Deep Feature Selection (DFS):** This technique involves adding a sparse layer with one-to-one connections between the input FC features and the first hidden layer of a multilayer perceptron. By weighting each feature, the method effectively selects a critical subset of functional connections that are most discriminative, simultaneously improving model performance and providing direct feature importance scores.[33]
2. **SHAP (Shapley Additive Explanations):** Based on cooperative game theory, SHAP calculates the contribution of each feature to a specific prediction. SHAP values are highly valuable as they offer both global insights (overall feature importance) and local explanations (why a specific individual was classified as ASD), aligning with the need for individualized diagnostic explanations.[32]

Furthermore, for analyzing dynamic features, methodologies like Layer-Wise Relevance Propagation (LRP) combined with time-window sliding techniques can provide temporal interpretation, revealing *when* during the scan a particular set of functional connections contributed most to the classification decision.[34]

The convergence between XAI results and established neurobiological theory is the ultimate validation of interpretability. For instance, XAI applied to ABIDE data revealed that classifier decisions were heavily driven by regions within the Default Mode Network (DMN), aligning with biophysical simulations modeling regional alterations in Excitation/Inhibition (E/I)

balance—a hypothesized mechanism in psychiatric disorders.[21] This convergence provides robust computational support for mechanistic theories of ASD.

B. Identifying Reproducible Neurobiological Signatures

XAI and advanced feature selection techniques applied to the ABIDE dataset have successfully identified several reproducible neurobiological signatures associated with ASD, providing clear targets for model interpretation:

1. **Default Mode Network (DMN):** Regions consistently identified as most critical for differentiating ASD from neurotypical controls include the **Posterior Cingulate Cortex (PCC)** and the **Precuneus**, both central nodes in the DMN.[21] Atypical temporal dependencies within the DMN are potential biomarkers that LSTM models are optimized to detect.[20]
2. **The Cerebellar Imperative:** The cerebellum, often excluded or poorly analyzed in standard FC protocols, has been shown to contain reproducible putative biomarkers for accurate ASD diagnosis.[36] Specifically, discriminatory connections lie between the cerebellum and motor areas, as well as between the cerebellum and the frontal cortex. These connections are hypothesized to relate to sensory processing and social behavior, which are known to be altered in ASD.[36] It is critically important that the analysis pipeline, including atlas selection, ensures robust coverage and feature extraction of cerebellar connectivity.
3. **Widespread Connectivity Alterations:** Feature selection studies consistently indicate that the ASD group exhibits a significantly higher number of **weak functional connections** spread across the cerebral hemisphere compared to typically developed controls.[33]

VI. Synthesis: Actionable Roadmap for Successful ASD Neuroimaging Research

To overcome the challenges of ABIDE heterogeneity and r-fMRI ambiguity, and to achieve explainable ML models based on a statistically defined "normal threshold," an integrated, multi-stage pipeline is required.

A. The Recommended Robustification Pipeline

The following six steps represent the necessary sequence for developing robust and interpretable ASD biomarkers from multi-site rs-fMRI data:

1. **Preparation and Quality Control:** Implement rigorous motion correction utilizing a parsimonious model (6 MPs) combined with **volume interpolation** to preserve the temporal continuity of the $4D$ time series.
2. **Connectivity Extraction and Feature Stability:** Extract Functional Connectivity (FC) features using a high-granularity **functional atlas** (e.g., Glasser or Schaefer $400+$ nodes). Empirically test both the filt_noglobal and filt_global preprocessing pipelines, selecting the strategy that maximizes discriminative performance after harmonization, while acknowledging the interpretation constraints of GSR-induced anti-correlations.
3. **Mandatory Harmonization:** Apply the **ComBat** technique to the high-dimensional FC features (static, dynamic state metrics, or graph metrics). Quantitatively validate that ComBat successfully removes site effects while preserving associations with biological covariates.
4. **Baseline Definition via Normative Modeling:** Establish the "normal threshold" by training a normative model on the harmonized TD data. Generate individualized **Deviation Z-scores** for every FC feature in both the TD and ASD groups.
5. **ML Training on Individualized Features:** Train advanced temporal classifiers (e.g., LSTMs, utilizing sliding window data) or Graph Convolutional Networks (GCNs) using the personalized **deviation Z-scores** as input features. This shifts the diagnostic focus to detecting patterns of individualized abnormality.
6. **Interpretation and Validation:** Apply XAI methods (e.g., Integrated Gradients, DeepLift, or SHAP) to the trained classifier to identify the most discriminative FC features. Validate the XAI findings by confirming that the identified features align with known neurobiological markers of ASD, particularly within the DMN (PCC/Precuneus) and the **Cerebellum**.

B. Future Directions for Convergent Validation

For future research, achieving convergent evidence across modalities will further solidify the neurobiological interpretability of the findings. Integrating functional connectivity data with structural connectivity metrics derived from Diffusion Tensor Imaging (DTI) can significantly enhance accuracy.[37] For example, integrating DTI features related to the frontal lobe and corpus callosum with fMRI features has demonstrated very high classification accuracies.[37]

Finally, given the established heterogeneity of ASD, the most fruitful approach for clinical

translation lies in leveraging the personalized deviation maps generated by the normative model to identify and classify distinct ASD functional subtypes.[29] Focusing ML efforts on these defined subtypes, rather than the broad, heterogeneous ASD spectrum, holds the greatest potential for defining highly penetrant connectivity signatures necessary for individualized precision medicine.[30]

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