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Research Article

Determination of Sensitive Frequency Bands for Cytotoxicity Analysis Using Microwave Dielectric Measurements

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ABSTRACT

This study presents a comprehensive frequency-resolution analysis framework to identify biologically significant frequency bands for cancer cell monitoring in the 10–20 GHz range. By integrating statistical significance, effect size, dielectric deviation magnitude, and temporal stability analysis, the spectral response of cell cultures exposed to cytotoxic agents was systematically evaluated. The results revealed a dual-optimization environment: the 13.9–14.2 GHz range showed a statistically significant global maximum for signal coherence, while higher frequencies demonstrated superior dielectric contrast due to γ-dispersion mechanisms. To resolve this balance, a composite sensitivity score and band-averaged analysis were used. The evaluation identified the 16–18 GHz band as the optimum working region, offering significantly enhanced physical contrast and temporal robustness while maintaining a composite score almost identical to the statistical peak (<0.7% difference). This study demonstrates that data-driven, multi-criteria frequency selection provides a robust foundation for sensor design and paves the way for the development of highly sensitive, frequency-selective microwave biosensors for toxicological screening.

1. Introduction

Dielectric properties are fundamental electromagnetic parameters that define a material's ability to polarize in the face of an electric field [1]. Dielectric values can also provide valuable information about the electromagnetic behavior of biological materials, enabling the assessment of physiological changes in cell cultures exposed to cytotoxic agents [2], [3]. Microwave biosensors have emerged as a powerful, label-free technique for the dielectric characterization of biological materials, offering advantages such as non-invasive measurement, real-time monitoring capability, and the elimination of chemical reagents. Unlike optical or electrochemical biosensors, microwave-based approaches examine changes in the electromagnetic properties of biological samples; these properties are closely related to cell composition, membrane integrity, intracellular structure, and the physicochemical properties of the surrounding environment. It has been demonstrated that biological tissues and cell cultures exhibit a strong interaction with electromagnetic fields, which is frequency dependent. As frequency increases, the effective permeability of biological media decreases due to the gradual suppression of polarization mechanisms such as ionic conduction, interfacial polarization, and dipolar relaxation [4], [5]. This dispersion behavior, often referred to as dielectric dispersion, forms the physical basis for microwave sensing of biological systems in the frequency domain. Recent advances in microwave biosensors have demonstrated diverse applications, from cancer cell detection to the determination of dielectric properties of cell suspensions. In particular, transmission line-based sensors have demonstrated the ability to extract frequency-dependent

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dielectric parameters of cell lines [6], [7]. Furthermore, the integration of microfluidic platforms with microwave biosensors has enabled controlled, reproducible, and real-time dielectric characterization of biological samples across a wide frequency range. To develop biosensors that offer all these advantages, it is essential to first understand the dielectric properties of biological suspensions. Since dielectric properties vary with frequency, dielectric measurement over a wide frequency range is crucial. The need to know the frequency value to be used during biosensor design has led to the necessity of determining the appropriate frequency based on the measured dielectric properties [8], [9].

Despite the availability of wideband measurement capabilities, many microwave biosensing studies rely on resonant structures operating at a single frequency or a narrow frequency band. The choice of operating frequency is usually determined by sensor geometry rather than data-driven sensitivity analysis [10], [11].

Due to the inherently distributed nature of biological dielectric properties, it is unlikely that a single fixed frequency will universally capture all cytotoxicity-related effects. This study proposes a statistical frequency-response mapping framework to independently identify frequency regions that maximize cytotoxic susceptibility by integrating statistical, multivariate, and data-driven analytical approaches.

2. Literature Review

2.1. Dielectric Properties of Biological Samples

Biological samples can be modeled as complex dielectric systems with frequency-dependent permittivity arising from multiple polarization mechanisms, including ionic conduction at low frequencies, interfacial polarization at medium frequencies, and dipole relaxation at microwave frequencies. Dielectric spectroscopy consistently reports that permittivity (ε') decreases with increasing frequency [1], [12], [13], [14]. Planar microwave sensors and transmission line structures provide information on various aspects based on the change in dielectric properties of cell suspensions. They show strong correlations between frequency-dependent permittivity and biological parameters such as cell concentration and viability. These findings confirm that dielectric variation contains information that can be used for sensing applications [15], [16].

2.2. Dielectric Property Measurement of Biological Samples Using VNA-Based Coaxial Probe Techniques

Dielectric characterization of biological materials is commonly performed using vector network analyzer (VNA)-based measurement techniques, which enable accurate and broadband extraction of complex permittivity over microwave frequencies. Among these methods, the open-ended coaxial probe technique has become a widely accepted standard for measuring the dielectric properties of liquids, soft tissues and cell suspensions due to its non-destructive nature, minimal sample preparation requirements and suitability for broadband frequency analysis.

In the coaxial probe method, an open-ended coaxial line is brought into direct contact with the material under test and the reflected signal (S_{11}) is measured using a VNA. The interaction between the fringing electromagnetic fields at the probe tip and the sample causes changes in the reflection coefficient, which can be related to the complex permittivity of the material through well-established electromagnetic models [17]. This approach allows simultaneous extraction of the real (ε') and imaginary (ε'') components of permittivity across a wide frequency range. The most important contribution of coaxial probe measurements is that we can measure the variation of the absolute ε' value at different frequencies [18], [19].

Numerous studies have validated the accuracy and reliability of coaxial probe techniques for biological dielectric measurements, particularly in the microwave frequency range. Previous investigations have reported successful dielectric characterization of cell suspensions, tissue-mimicking phantoms and biological fluids using this method, demonstrating strong agreement with theoretical dispersion models and alternative measurement techniques [20]. The broadband nature of VNA-based measurements makes the coaxial probe particularly well suited for studying dispersive biological media where dielectric properties vary significantly with frequency [21].

Compared to resonant or narrowband microwave sensing approaches, coaxial probe measurements provide continuous frequency-domain data without being constrained by sensor geometry or resonance conditions. This characteristic is especially advantageous for studies aimed at frequency optimization as it enables post-measurement analysis of dielectric responses over the entire frequency spectrum. As a result, the operating frequency can be selected based on data-driven sensitivity and statistical significance rather than predetermined design constraints [22].

In the context of cytotoxicity assessment, dielectric property changes induced by cell membrane disruption, intracellular leakage and variations in viable cell concentration manifest as frequency-dependent variations in permittivity. VNA-based coaxial probe measurements therefore offer a robust experimental foundation for investigating cytotoxic effects through dielectric spectroscopy and for identifying frequency regions that maximize sensitivity to such biological alterations [8], [20].

2.3. Microwave Methods in Cytotoxicity Assessment

Traditional cytotoxicity assessment techniques provide endpoint quantification of cell viability but do not readily support real-time or label-free monitoring. Recent research has explored microwave biosensors as an alternative, demonstrating capability to detect cell viability changes by observing changes in scattering parameters. Resonator-based methods have been validated against conventional biochemical assays, indicating that microwave measurements can reflect cytotoxic effects. Nonetheless, these studies typically focus on discrete frequency points or narrow bands, leaving a systematic frequency optimization largely unexplored [23], [24], [25], [26].

2.4. Statistical and Multivariate Analysis in Dielectric Sensing

Multivariate techniques such as Principal Component Analysis (PCA) and statistical significance testing have been applied in dielectric sensing to manage complex datasets and distinguish subtle differences in frequency responses. PCA enables identification of dominant variance components across frequency features, aiding in feature selection for classification tasks. When combined with metrics like effect size (Cohen's d) and ANOVA, these methods provide robust frameworks to quantify the significance of observed differences between sample groups. Integrating such analytical tools in microwave biosensing facilitates more objective frequency selection and discrimination assessment than reliance on single-frequency heuristics [27], [28], [29], [30], [31], [32].

3. Materials and Methods

3.1. Data Acquisition

The dielectric data analyzed in this study were obtained by coaxial probe measurements in the 10-20 GHz range on cultured cell samples exposed to different cytotoxic agent concentrations over multiple time intervals. A Vector Network Analyzer (VNA) was set to the 10-20 GHz frequency range, and a coaxial probe was connected. Measurements were performed after calibrating the coaxial probe [22]. The control and multiple treated groups (e.g., 10 μg/ml to 1000 μg/ml at 24, 48, and 72 hours) provided a comprehensive dataset for frequency analysis. The measurement setup prepared with VNA and coaxial probe is shown in Figure 1.

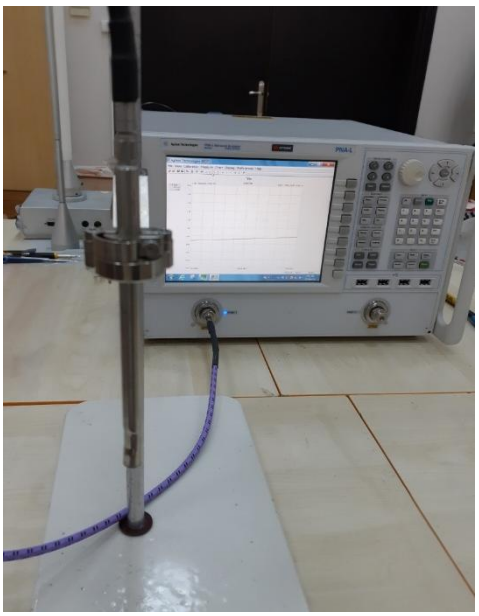

Figure 1. VNA and Coaxial Probe Measurement

3.2. Data Structure and Preprocessing

The dataset consists of frequency-dependent true dielectric constant values measured with a frequency resolution of 7.5 MHz between 10 and 20 GHz. Measurements were obtained for cell cultures exposed to four different cytotoxic agent concentrations (10, 100, 500, and 1000 μg/ml) for three incubation periods (24, 48, and 72 hours), as well as for control and medium-only samples. The obtained data were used to create a multidimensional dataset capturing both dose- and time-dependent cytotoxic effects across the microwave frequency spectrum. Before analysis, the dielectric constant spectrum of the control sample was used as the primary reference. For each frequency point, the difference between the processed samples and the control was calculated to highlight dielectric changes due to cytotoxicity and suppress systematic measurement biases. No smoothing or filtering was applied to the frequency responses to preserve the original spectral characteristics and prevent artificial enhancement of specific frequency regions.

3.3. Software Implementation and Data Processing

All data processing, statistical analysis, and machine learning evaluations were performed using software developed in Python. The entire analysis pipeline was designed and implemented by the authors to provide complete control over data processing, feature extraction, and frequency-resolution evaluation, while maintaining transparency and reproducibility of results. Raw dielectric measurement data obtained from the vector network analyzer were first imported into the Python environment and organized into structured datasets according to experimental conditions, including cytotoxic agent concentration and control groups. Preprocessing steps included frequency alignment, consistency checks between repeated measurements, and normalization

procedures as needed to minimize systematic measurement variations while preserving biologically relevant dielectric differences. The Python-based nature of the system enabled the seamless integration of various methods within a unified pipeline. This strategy ensured methodological consistency between analyses and allowed for iterative improvement of evaluation criteria. The modular structure of the codebase facilitates the extension of the framework to other cell lines, cytotoxic agents, and sensor configurations, thereby supporting future comparative and longitudinal studies.

3.4. Statistical Frequency Response Mapping and Data-Driven Feature Extraction

The dielectric response of biological samples exposed to cytotoxic agents exhibits complex, frequency-dependent behavior resulting from distributional polarization mechanisms and cellular changes. Susceptibility to cytotoxic effects cannot be reliably inferred from a single frequency point or a single analytical measurement. To overcome this challenge, a comprehensive statistical frequency response mapping framework was used to systematically measure frequency-dependent susceptibility, robustness, and discriminative ability. For each frequency point within the 10-20 GHz measurement band, multiple complementary statistical and multivariate measurements were calculated. Each measurement identified a different aspect of the relationship between cytotoxicity-induced dielectric variation and frequency. Microwave biosensors typically rely on measurements at an operating frequency determined by sensor geometry or resonance conditions. However, biological samples exhibit different behaviors at different frequencies. Focusing on a single frequency can lead to missing other frequency values where more effective results could be obtained. Therefore, each frequency point was treated as an independent detection channel, and frequency selection was performed entirely data-driven, without assumptions.

3.4.1. Mean dielectric deviation (Δε') relative to control sample

The mean dielectric constant difference between samples exposed to the cytotoxic agent and the control group was calculated at each frequency point. This metric represents the average magnitude of dielectric degradation caused by cytotoxic exposure and serves as a direct indicator of biological change. Since cytotoxic effects such as membrane degradation, intracellular leakage, and changes in viable cell density alter the effective permeability of the medium, $\Delta\varepsilon'$ provides a physically interpretable measure of the cytotoxic response. However, mean dielectric deviation alone does not account for biological variability and measurement noise. Therefore, complementary statistical measures are needed for an effective frequency assessment.

3.4.2. Frequency-dependent variability and noise characterization

To quantify measurement variability, the standard deviation of the dielectric constant values of the samples was calculated at each frequency. Variability in microwave dielectric measurements can be caused by factors such as the predominance of ionic conduction at low frequencies, probe-sample contact variations, and biodiversity. The inclusion of this metric is critical; large average dielectric deviations combined with high variability may not provide reliable discrimination. Therefore, the standard deviation serves as a normalization and confidence indicator when evaluating frequency sensitivity.

3.4.3. Effect size analysis

An effect size measure was calculated at each frequency point to quantify the magnitude of the separation between samples exposed to the cytotoxic agent and the control sample. Effect size provides a scale-independent measure of resolving power, allowing comparisons between frequencies independently of absolute transmittance magnitude. Unlike statistical significance tests, effect size is insensitive to sample size and directly reflects the practical significance of the observed dielectric differences. Frequencies showing large effect sizes indicate strong and consistent dielectric changes due to cytotoxicity. Unlike p-value–based significance testing, effect size directly reflects practical discrimination strength and remains meaningful even in datasets with limited sample size, which is typical for biological experiments.

3.4.4. Separation index based on signal-to-noise ratio

The separation index is defined as the absolute mean dielectric deviation normalized to the relevant standard deviation. This measure clearly captures the signal-to-noise ratio of the cytotoxic response at each frequency point. The separation index favors frequencies where dielectric changes due to cytotoxicity are both qualitatively large and stable throughout the experimental conditions. This is particularly important for real-world biosensor applications where robustness and reproducibility are as critical as accuracy.

3.4.5. Frequency resolution statistical significance test (ANOVA)

One-way analysis of variance (ANOVA) was performed independently at each frequency point to assess whether the differences in dielectric constant between cytotoxic agent-treated samples and control samples were statistically significant. This frequency-resolution ANOVA approach allows for the identification of adjacent frequency regions where cytotoxic effects consistently exceed random variability. Instead of relying on isolated p-values, statistical significance was analyzed across the entire frequency spectrum to identify frequency bands of

biological significance. Statistical significance was interpreted using effect size and variability measures to avoid overemphasizing isolated frequency points with limited practical importance.

3.4.6. Multivariate frequency additive analysis with PCA

Principal Component Analysis (PCA) was applied to the transposed dielectric constant dataset, with each frequency point treated as a feature and each experimental condition as an observation. PCA decomposes the dataset into orthogonal components that capture dominant sources of variance. Absolute loadings of the first principal component were used to measure the contribution of each frequency point to the variance associated with dominant cytotoxicity. Frequencies with high PCA loadings are those that contribute most strongly to distinguishing cytotoxic conditions, providing an independent multivariate perspective on the significance of frequency. PCA provides an independent multivariate validation of frequency relevance by capturing correlated spectral behavior that may not be evident from univariate analyses alone.

3.4.7. Composite frequency optimization score

To integrate the complementary information provided by individual measurements, a composite frequency optimization score was constructed. Each measurement (mean dielectric deviation, effect size, discrimination index, ANOVA significance, and PCA loading) was normalized to a common scale and summed up to obtain a composite sensitivity score for each frequency point. This composite approach reduces biases specific to any single measurement and ensures that selected frequency regions consistently exhibit high performance across multiple analytical dimensions. Frequency regions that ranked highest by the composite score were considered optimal working bands for cytotoxicity assessment using microwave dielectric sensing. This composite framework ensures that selected frequency bands are not optimized for a single analytical criterion but represent a balanced convergence of physical contrast, statistical robustness, and multivariate relevance.

4. Results and Discussion

4.1. Frequency-Dependent Dielectric Response to Cytotoxic Exposure

Under all experimental conditions, the real part of the dielectric constant (ε′) exhibited a gradual decrease with increasing frequency in the 10–20 GHz range. This behavior is consistent with the characteristic dielectric dispersion of biological media in the microwave band, where γ-dispersion (gamma-dispersion) mechanisms driven by the dipolar relaxation of water molecules are dominant. However, relative to the control group, samples exposed to cytotoxic agents displayed systematic deviations in ε′. Figure 2 illustrates the frequency-dependent dielectric deviation (Δε′) relative to the control reference. Notably, the deviation values are negative, indicating a reduction in the dielectric constant of the treated samples. The magnitude of this deviation was not uniform across the spectrum. While relatively modest deviations were recorded in the lower frequency region (<12 GHz, Δε′≈-1.4), the difference became significantly more pronounced at higher frequencies, reaching a maximum magnitude around 20 GHz (Δε′≈-2.0). This trend indicates that the cytotoxic-induced alterations in cellular hydration and structural integrity are more effectively resolved at higher microwave frequencies, resulting in increased dielectric contrast.

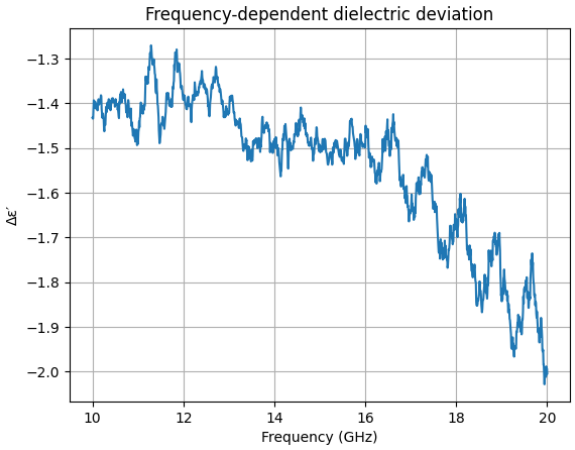

Figure 2. Mean Dielectric Deviation (Δε′) versus Frequency for all Cytotoxic Doses

4.2. Measurement Variability and Frequency Stability

Analysis of Epsilon's frequency-dependent standard deviation reveals significant variability trends across the measured spectrum. In the lower frequency range (10–14 GHz), a downward trend in variability is observed, reaching a minimum of 1.19 standard deviations around 14 GHz, representing the region of highest measurement stability. Conversely, variability increases significantly in the higher frequency band (14–20 GHz), exceeding 1.50 at 20 GHz. This trend indicates that measurement accuracy is optimal around 14 GHz, but signal stability deteriorates at higher frequencies. Possible reasons for this include instrument limitations or increasing scattering effects as the frequency increases. The variation of measurement variability with frequency is shown in Figure 3.

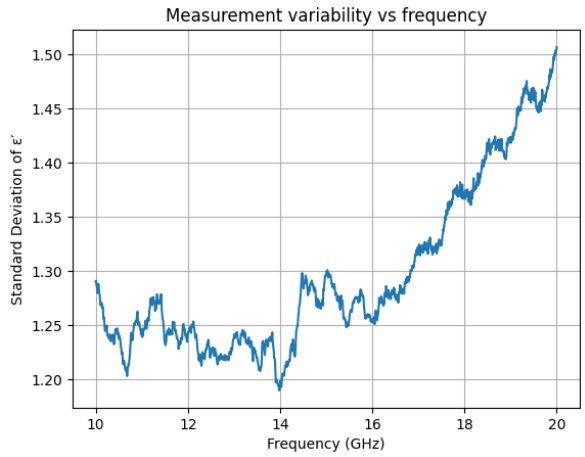

Figure 3. Frequency-Dependent Standard Deviation of ε′ Across Experimental Conditions

4.3. Statistical Effect Size and Frequency-Specific Discrimination

Effect size (η^2) analysis was performed to measure the magnitude of the distinction between cytotoxic agent-treated and control samples, independent of sample size. The results revealed a non-monotonic frequency dependence. The results are given in Figure 4. Although the global maximum effect size was observed towards 20 GHz, this region was previously identified as having high measurement instability. Consequently, attention was directed to the distinct local peak observed at around 13.53 GHz. While this frequency represented a prominent point of distinction within the more stable frequency band, it did not perfectly coincide with the absolute minimum variability observed at 14 GHz. This discrepancy—where the optimal sensitivity (effect size) and optimal stability do not align—highlights the inherent limitation of relying on a single statistical measure and underscores the necessity for a multi-criteria evaluation framework.

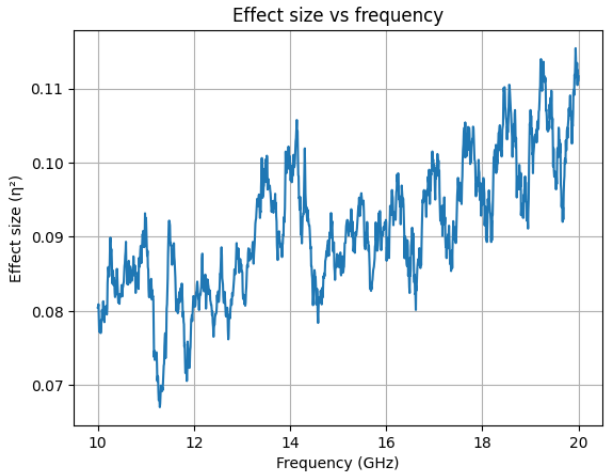

Figure 4. Effect Magnitude versus Frequency (η^2) with Highlighted Global Maximum

4.4. Temporal Robustness of Frequency Sensitivity

Time-resolved analysis over 24-hour, 48-hour, and 72-hour exposure periods revealed that frequency sensitivity is governed by both dose- and time-dependent dynamics. While certain frequencies exhibited high sensitivity at single time points, they lacked consistency across the full incubation duration. Figure 5 shows the temporal stability heat map, illustrating the variation of the average dielectric constant (ε′) over time. Notably, the vertical uniformity observed in the heatmap confirms that the dielectric dispersion profile remains stable against temporal biological variability. Within this stable landscape, a frequency band centered around 16.67 GHz was identified as optimal. This band not only maintains a consistent dielectric response across all exposure periods but also coincides with significant dielectric deviation. Such temporal robustness is critical for longitudinal monitoring, ensuring that the observed contrast is driven by cytotoxicity rather than temporal fluctuations in the measurement baseline. This result demonstrates that temporal stability constitutes a distinct optimization criterion, independent of purely statistical separation measures.

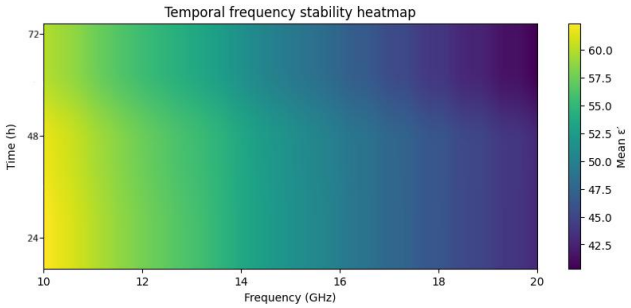

Figure 5. Time-Resolved Sensitivity Heat Map

4.5. Dose-Response Behavior and Monotonic Trend Analysis

Spearman rank correlation analysis was performed to evaluate the monotonic dose-response behavior across the frequency spectrum. The strongest monotonic relationships between cytotoxic substance concentration and dielectric response were observed in the upper frequency region of 18–20 GHz, driven by the dominant γ-dispersion contrast. Although this region exhibited clear dose-dependent trends, the increased variability compared to the mid-frequencies limited its overall discriminative robustness. In contrast, the 16–18 GHz band maintained a strong monotonic correlation comparable to the upper band, but with significantly better signal stability. These results demonstrate that monotonicity alone does not guarantee optimum detection performance and should be evaluated in conjunction with stability and composite susceptibility measures, further supporting the selection of the 16–18 GHz band.

4.6. Multivariate and Machine Learning-Based Validation

Multivariate analysis and machine learning were used as complementary validation tools rather than primary

optimization drivers. Binary classification between cytotoxic and non-cytotoxic samples achieved moderate accuracy, with support vector machines showing the highest performance among the tested models. Interestingly, purely data-driven Machine Learning models tended to highlight frequency regions around 11–12 GHz, where global variance is relatively high among samples. However, these frequencies did not fully overlap with the optimal regions identified by the proposed statistical stability and composite sensitivity framework (14–18 GHz). This discrepancy highlights a critical limitation of data-driven approaches: they may prioritize features with high variance. Consequently, while ML confirms measurable differences exist, the divergence in feature selection underscores the necessity of the proposed physics-aware statistical framework for reliable frequency optimization, rather than relying solely on automated feature selection.

4.7. Integrated Interpretation and Optimal Frequency Band Concept

The combined analyses demonstrate that no single frequency simultaneously maximizes all performance criteria. As shown in Figure 6, the composite optimization profile, the mathematical global maximum for the sensitivity score is observed in the 13.8–14.2 GHz range. However, relying solely on this peak would compromise the magnitude of dielectric deviation, which is superior at higher frequencies. To resolve this, the integrated framework prioritizes a region that balances score magnitude with high dielectric contrast. Consequently, the evaluation identifies the 16–18 GHz band—specifically the plateau region around 17 GHz—as the optimal operational domain. Unlike the narrow peak at 14 GHz, this band offers a broad, high-performance plateau (Composite Score > 2.5) that coincides with stronger dielectric separation capabilities. This finding supports a band-based optimization strategy, where the 16–18 GHz range provides the necessary trade-off between statistical sensitivity (effect size), signal magnitude (deviation), and operational robustness.

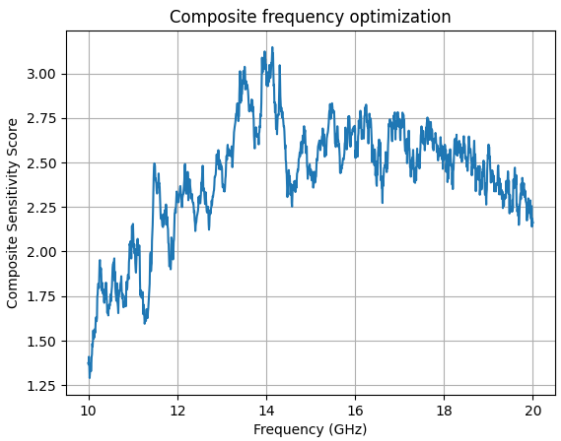

Figure 6. Composite Sensitivity Score vs Frequency

4.8. Quantitative Band Analysis and Operational Frequency Selection

The optimization phase was subjected to a dual-level analysis by determining specific peak frequencies and evaluating band-averaged performance. As detailed in Table 1, discrete frequency analysis reveals that the absolute mathematical maximums of the Composite Sensitivity Score cluster in a narrow range of 13.9–14.2 GHz, with peak scores exceeding 3.10.

A thorough review of Table 1 reveals that the 14.14 GHz frequency attained the maximum global sensitivity score (3.147). This enhanced performance is attributable to a high Separation Index (1.290) and a substantial Effect Size (η^2= 0.105), which collectively signify that the dielectric alterations induced by cellular toxicity are most discernible from random biological variations within this restricted spectral window. This confirms that, in terms of statistical signal-to-noise ratio, the 14 GHz region offers the highest stability due to its minimal standard deviation (STD≈1.21), representing the most repeatable measurement point across all experimental conditions. Sensors to be developed for detecting biological samples require operation over a stable bandwidth rather than a single frequency point. As illustrated in Table 2, the band-averaged sensitivity analysis reveals that the performance distribution assumes a broad plateau configuration rather than a pronounced peak. The average composite score for the 14–16 GHz band is 2.625, while the 16–18 GHz band reaches a nearly identical score of 2.609. In contrast, the lower frequency band (10–12 GHz) demonstrates significantly lower sensitivity with an average score of 1.895, highlighting that early microwave frequencies are less effective at capturing subtle cytotoxic alterations. This represents a negligible performance drop of less than 0.7%. This finding is critical for the final frequency selection. While the 14 GHz region demonstrates theoretical peak, shifting the operation to the 16-18 GHz band offers a substantial benefit in the form of a higher dielectric deviation magnitude, as previously illustrated in Figure 2, without incurring a significant decline in the composite sensitivity score. Quantitatively, while the 14 GHz region is statistically the most stable, the 16–18 GHz region provides a superior biophysical signal due to the enhanced contrast provided by γ-dispersion mechanisms at higher frequencies. Consequently, the 16-18 GHz band is identified as the operational optimum, as it maximizes the biophysical contrast mechanisms that are dominant at higher frequencies while effectively preserving the high composite performance of the 14 GHz region.

Table 1. Top Sensitive Frequencies

Frequency	Delta_eps	STD	Eta_sq	SepIndex	p_value	CompositeScore	Band
14.14	-1.563646	1.211636	0.105751	1.290524	0.240813	3.147233	(14, 16]
13.9675	-1.511128	1.193667	0.10217	1.265954	0.24933	3.123205	(12, 14]
14.155	-1.551252	1.211761	0.104236	1.280163	0.244376	3.113538	(14, 16]
14.1325	-1.554479	1.212474	0.104515	1.282072	0.243716	3.096612	(14, 16]
13.9075	-1.50973	1.197003	0.101491	1.261259	0.250985	3.088322	(12, 14]
14.1475	-1.551722	1.216567	0.103556	1.275492	0.245996	3.087406	(14, 16]
13.9825	-1.494945	1.189844	0.100792	1.256421	0.252699	3.081312	(12, 14]
13.96	-1.501478	1.194826	0.100825	1.25665	0.252618	3.078483	(12, 14]
13.975	-1.50228	1.194823	0.100922	1.257324	0.252378	3.070754	(12, 14]
13.9525	-1.496495	1.194684	0.100245	1.252628	0.254049	3.064199	(12, 14]

Table 2. Band-Averaged Composite Sensitivity

Band	Average CompositeScore
(10, 12]	1.895577
(12, 14]	2.535935
(14, 16]	2.624983
(16, 18]	2.608529
(18, 20]	2.415831

5. Conclusions

In this study, a comprehensive frequency-resolution analysis framework was developed to investigate cytotoxic effects in cancer cell cultures using microwave dielectric measurements. Unlike traditional approaches relying on single-frequency interrogation or global spectral descriptors, the proposed methodology systematically assesses the dielectric response across a broad microwave frequency range and integrates statistical, multivariate, and machine learning-based analyses to identify biologically significant frequency regions. The results clearly demonstrate that cytotoxic exposure causes measurable and frequency-dependent changes in the effective dielectric properties of cell cultures. Statistical frequency response mapping revealed that these changes are not uniformly distributed across the spectrum. The analysis identified a dual-optimization environment: the 13.9–14.2 GHz range showed a statistically significant global maximum in terms of signal coherence and effect size, while the magnitude of dielectric deviation was found to increase at higher frequencies due to γ-dispersion mechanisms. A critical finding of this study is the resolution of this balance problem through band-averaged sensitivity analysis. Although the 14 GHz region theoretically presents the statistical peak, the 16-18 GHz band has been identified as the optimal operating range. Integrated analysis has shown that this band maintains a composite sensitivity score nearly identical to the global maximum (with a negligible difference of <0.7%) and offers superior dielectric contrast and temporal robustness. This confirms that long-term or repeated measurements can be performed in the 16-18 GHz range without compromising measurement consistency, a crucial requirement for practical monitoring systems. Multivariate analysis using principal component analysis confirmed that a limited subset of frequency points dominates the overall variance associated with cytotoxic exposure. This finding supports the hypothesis that broadband microwave measurements contain redundant information, and that biologically relevant insights can be obtained by focusing on statistically optimized frequency bands rather than the entire spectrum. While machine learning confirmed the classify-ability of the dataset, the distinct feature selection results highlighted the risk of purely variance-driven approaches. This reinforces the value of the proposed statistical framework in identifying biophysically relevant and stable frequency bands that algorithms might overlook. Overall, the presented framework demonstrates that microwave dielectric spectroscopy, when combined with rigorous frequency-resolution statistical analysis, is an effective method for precise frequency selection in biosensor design. The findings pave the way for the development of compact, frequency-selective microwave biosensors capable of rapid and label-free cytotoxicity screening, with potential applications in drug testing, toxicological studies, and real-time biological monitoring. Future studies could focus on extending this approach to different cell lines and sensor geometries, particularly by integrating the identified 16-18 GHz optimal band into resonant sensor architectures to further enhance sensing performance.

Declaration of Ethical Standards

The article does not contain any studies with human or animal subjects.

Credit Authorship Contribution Statement

Taha Fatih Ateş: Conceptualization, Design, Software, Resources, Writing – review and editing. Ali Osman Özkan: Resources, Methodology, Validation, Project

administration, review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] C. Gabriel, S. Gabriel, and E. Corthout, "The dielectric properties of biological tissues: I. Literature survey," *Phys. Med. Biol.*, vol. 41, no. 11, pp. 2231–2249, Nov. 1996, doi: 10.1088/0031-9155/41/11/001.

[2] R. Pethig, "Dielectric properties of biological materials: Biophysical and medical applications," *IEEE Trans. Electr. Insul.*, vol. EI-19, no. 5, pp. 453–474, Oct. 1984, doi: 10.1109/TEI.1984.298769.

[3] F. Artis, D. Dubuc, J.-J. Fournié, M. Poupot, and K. Grenier, "Microwave dielectric spectroscopy for biological cells suspensions analysis and proliferation evaluation," in *Proc. 44th Eur. Microw. Conf. (EuMC)*, Oct. 2014, pp. 275–278, doi: 10.1109/EuMC.2014.6986423.

[4] S. Sikiru, N. Yahya, H. Soleimani, A. M. Ali, and Y. Afeez, "Impact of ionic-electromagnetic field interaction on Maxwell-Wagner polarization in porous medium," *J. Mol. Liq.*, vol. 318, p. 114039, Nov. 2020, doi: 10.1016/j.molliq.2020.114039.

[5] B. Greenebaum and F. S. Barnes, Eds., *Bioengineering and Biophysical Aspects of Electromagnetic Fields*, 3rd ed. Boca Raton, FL, USA: CRC Press, 2018, doi: 10.1201/9781315221540.

[6] C. G. Juan, K. Grenier, M. H. Zarifi, A. Ebrahimi, and F. Martin, "Planar microwave sensors: State of the art and applications," *IEEE Access*, vol. 13, pp. 143985–144038, 2025, doi: 10.1109/ACCESS.2025.3599112.

[7] P. Mehrotra, B. Chatterjee, and S. Sen, "EM-wave biosensors: A review of RF, microwave, mm-wave and optical sensing," *Sensors*, vol. 19, no. 5, p. 1013, Jan. 2019, doi: 10.3390/s19051013.

[8] K. Heileman, J. Daoud, and M. Tabrizian, "Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis," *Biosens. Bioelectron.*, vol. 49, pp. 348–359, Nov. 2013, doi: 10.1016/j.bios.2013.04.017.

[9] G. S. Kulkarni and Z. Zhong, "Detection beyond the Debye screening length in a high-frequency nanoelectronic biosensor," *Nano Lett.*, vol. 12, no. 2, pp. 719–723, Feb. 2012, doi: 10.1021/nl203666a.

[10] G. Guarin, M. Hofmann, J. Nehring, R. Weigel, G. Fischer, and D. Kissinger, "Miniature microwave biosensors: Noninvasive applications," *IEEE Microw. Mag.*, vol. 16, no. 4, pp. 71–86, May 2015, doi: 10.1109/MMM.2015.2394024.

[11] S. Guha, F. I. Jamal, and C. Wenger, "A review on passive and integrated near-field microwave biosensors," *Biosensors*, vol. 7, no. 4, p. 42, Dec. 2017, doi: 10.3390/bios7040042.

[12] Y. S. Cho and S.-J. Gwak, "Novel sensing technique for stem cells differentiation using dielectric spectroscopy of their proteins," *Sensors*, vol. 23, no. 5, p. 2397, Jan. 2023, doi: 10.3390/s23052397.

[13] Y. Shi, X. Bai, J. Yang, X. Wu, and L. Wang, "Optimized measurement methods and systems for the dielectric properties of active biological tissues in the 10Hz-100 MHz frequency range," *Front. Physiol.*, vol. 16, Jan. 2025, doi: 10.3389/fphys.2025.1537537.

[14] F. D. Tehrani, M. D. O'Toole, and D. J. Collins, "Tutorial on impedance and dielectric spectroscopy for single-cell characterisation on microfluidic platforms: theory, practice, and recent advances," *Lab Chip*, vol. 25, no. 5, pp. 837–855, 2025, doi: 10.1039/D4LC00882K.

[15] T. F. Ateş and A. O. Özkan, "Metamaterial-based microwave sensors," in *Contemporary Research in Engineering*. Konya, Turkey: All Sciences Academy, 2025, pp. 140–154.

[16] W. Sun *et al.*, "Biosensor with microchannel for broadband dielectric characterization of nanoliter cell suspensions up to 110 GHz," *Biosensors*, vol. 14, no. 7, p. 327, Jun. 2024, doi: 10.3390/bios14070327.

[17] T. F. Ateş, "Hücre canlılık tespiti için iletim hattı tabanlı biyosensör tasarımı ve uygulaması," Ph.D. dissertation, Dept. Elect. Electron. Eng., Necmettin Erbakan Univ., Konya, Turkey, 2025.

[18] A. La Gioia *et al.*, "Open-ended coaxial probe technique for dielectric measurement of biological tissues: Challenges and common practices," *Diagnostics*, vol. 8, no. 2, p. 40, Jun. 2018, doi: 10.3390/diagnostics8020040.

[19] C. Aydinalp, S. Joof, and T. Yilmaz, "Towards accurate microwave characterization of tissues: Sensing depth analysis of open-ended coaxial probes with ex vivo rat breast and skin tissues," *Diagnostics*, vol. 11, no. 2, p. 338, Feb. 2021, doi: 10.3390/diagnostics11020338.

[20] M. Hussein, F. Awwad, D. Jithin, H. El Hasasna, K. Athamneh, and R. Iratni, "Breast cancer cells exhibits specific dielectric signature in vitro using the open-ended coaxial probe technique from 200 MHz to 13.6 GHz," *Sci. Rep.*, vol. 9, no. 1, p. 4681, Mar. 2019, doi: 10.1038/s41598-019-41124-1.

[21] A. Šarolić and A. Matković, "Dielectric permittivity measurement using open-ended coaxial probe—Modeling and simulation based on the simple capacitive-load model," *Sensors*, vol. 22, no. 16, p. 6024, Jan. 2022, doi: 10.3390/s22166024.

[22] *Keysight 85070E Dielectric Probe Kit Technical Overview*, Keysight Technologies, Santa Rosa, CA, USA, 2017.

[23] S. K. Mahto, P. Chandra, and S. W. Rhee, "In vitro models, endpoints and assessment methods for the measurement of cytotoxicity," *Toxicol. Environ. Health Sci.*, vol. 2, no. 2, pp. 87–93, May 2010, doi: 10.1007/BF03216487.

[24] A. Amanati Shahri, A. H. Omidvar, G. Pamplona Rehder, and A. L. C. Serrano, "A microwave-based microfluidic cell detecting biosensor for biological quantification using the metallic nanowire-filled membrane technology," *Sensors*, vol. 22, no. 9, p. 3265, Jan. 2022, doi: 10.3390/s22093265.

[25] J.-M. Zhao *et al.*, "Microwave biosensor for the detection of growth inhibition of human liver cancer cells at different concentrations of chemotherapeutic drug," *Front. Bioeng. Biotechnol.*, vol. 12, May 2024, doi: 10.3389/fbioe.2024.1398189.

[26] S. Kamiloglu, G. Sari, T. Ozdal, and E. Capanoglu, "Guidelines for cell viability assays," *Food Frontiers*, vol. 1, no. 3, pp. 332–349, 2020, doi: 10.1002/fft2.44.

[27] Y. Lu, I. Cohen, X. S. Zhou, and Q. Tian, "Feature selection using principal feature analysis," in *Proc. 15th ACM Int. Conf. Multimedia (MM '07)*, New York, NY, USA, Sep. 2007, pp. 301–304, doi: 10.1145/1291233.1291297.

[28] C.-Y. J. Peng and L.-T. Chen, "Beyond Cohen's d: Alternative effect size measures for between-subject designs," *J. Exp. Educ.*, vol. 82, no. 1, pp. 22–50, Jan. 2014, doi: 10.1080/00220973.2012.745471.

[29] A. Malhi and R. X. Gao, "PCA-based feature selection scheme for machine defect classification," *IEEE Trans. Instrum. Meas.*, vol. 53, no. 6, pp. 1517–1525, Dec. 2004, doi: 10.1109/TIM.2004.834070.

[30] D. Kallogjeri and J. F. Piccirillo, "A simple guide to effect size measures," *JAMA Otolaryngol. Head Neck Surg.*, vol. 149, no. 5, pp. 447–451, May 2023, doi: 10.1001/jamaoto.2023.0159.

[31] M. Zhao, G. Downey, and C. P. O'Donnell, "Exploration of microwave dielectric and near infrared spectroscopy with multivariate data analysis for fat content determination in ground beef," *Food Control*, vol. 68, pp. 260–270, Oct. 2016, doi: 10.1016/j.foodcont.2016.03.031.

[32] T. F. Ateş and A. O. Özkan, "Evaluation of cytotoxicity effects by frequency response mapping," in *Proc. 6th Int. Conf. Eng. Nat. Soc. Sci.*, Konya, Turkey, Nov. 2025, pp. 160–162.