



## Dielectric Spectroscopy,

# a Yeast cell's perspective of dispersion dependent phenomena

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## INTRODUCTION

Recent development of electronic measuring equipment have enabled researchers, in particular the ones concerned with biological sample characterization, to further development in non-invasive and non-destructive techniques. Though the field of dielectric spectroscopy (DS) had already seen some research done before Maxwell's electromagnetic unification in 1873, effective research of the dielectric behavior applied to biological samples only was carried out in 1910 by Rudolf Höber, who provided first experimental evidence that cells possess a resistive dielectric membrane that surrounds a conducting electrolytic interior. Modern electronic measuring equipment alleviated requirements for research groups interested in such phenomena and in the mid-nineteenth century research done by H. P. Schwan provided empirical evidence that intracellular metabolism could be monitored by means of dielectric spectroscopy, sparkling numerous publications on the topic by many research groups. More recently new development is being made in some specific frontiers, spanning from miniaturization of DS measurements for individualized treatment of cells (cytometry); development of non-linear techniques that provide greater insight of intracellular metabolism; and the development of wideband techniques able to detect both extracellular metabolic elements and intracellular interactions. Alongside cancerous cells and bloods cells, yeast cells have been of great interest of the research community due to its structural similarities with animal cells but much simpler culture requirements. This work aims towards a brief overview of Dielectric Spectroscopy concept applied to biotechnology research and review the state of the art publications in the last 20 years, pointing out the differences between method applications and how frequency selection affects the desired object of study.

## METHODOLOGY

As can be extracted from Maxwell's equations and their accompanying constitutive relations, the presence of a material within a electromagnetic field creates distortions in its propagation. Propagation variation between materials, such as attenuation ( $\alpha$ ) and phase constant ( $\beta$ ) can be detected by appropriate transducers and with the correct mathematical treatment the fundamental electric constants can be extrapolated.

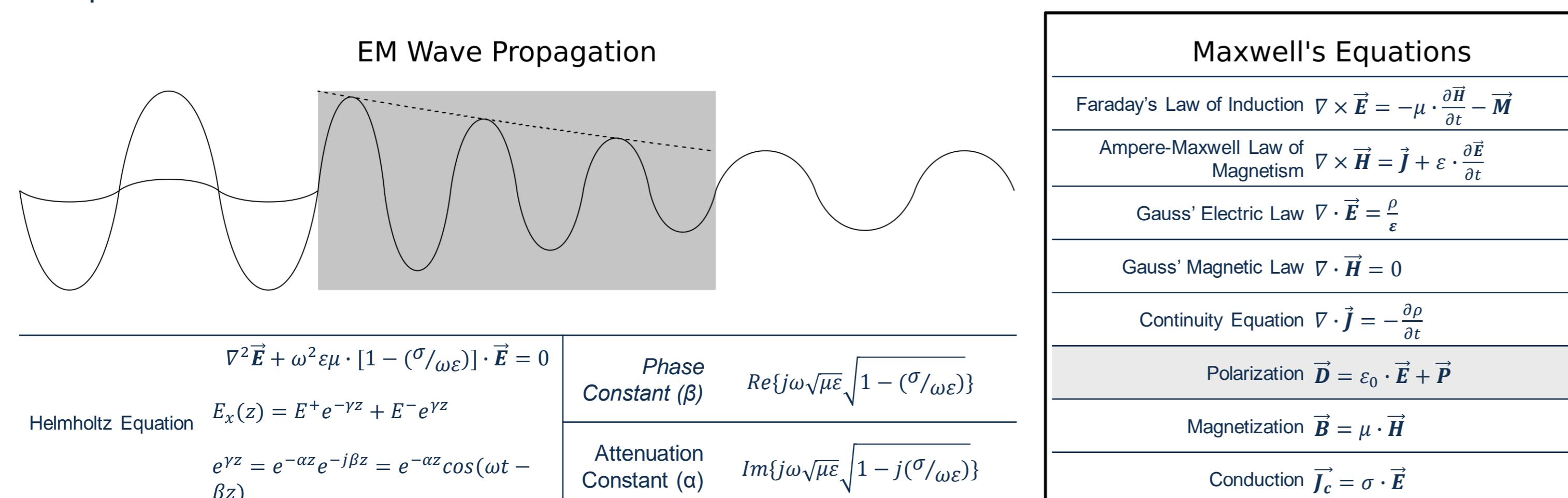


Figure 1 - EM propagation phenomena.

Source: Author.

Although Maxwell's equations gives us a simple and direct picture of electromagnetic propagation, determination of  $\epsilon$ ,  $\mu$  and  $\sigma$  prove to be a more ingenious task, as it relates both propagation in a atomic scale and in a macroscopic scale by means of the polarizability phenomena. Within such discussions for the correct values for the three constants, there are two key concepts that are fundamental for the understanding of the major issues in dielectric spectroscopy, both related to frequency dependence and the polarization mechanism, key concepts for this work.

The first concept is the oscillatory behavior of matter it self in time-harmonic electromagnetic fields. As first introduced by Paul Drude in 1900 and further developed by Hendrik A. Lorentz in 1905, the motion of electrons within a atomic arrangement was shown to closely obey oscillatory equations such as the classical mass-spring models. Although initially developed to describe the electronic behavior, this theory was later expanded to atomic, molecular and even to crystalline structures, as shown in Fig. 2. The key parameter that allowed for such wide range applicability was the different relaxation times attributed to each domain. As one might expect, smaller particle systems present smaller masses, and thus lower time constants when compared to more massive structures.

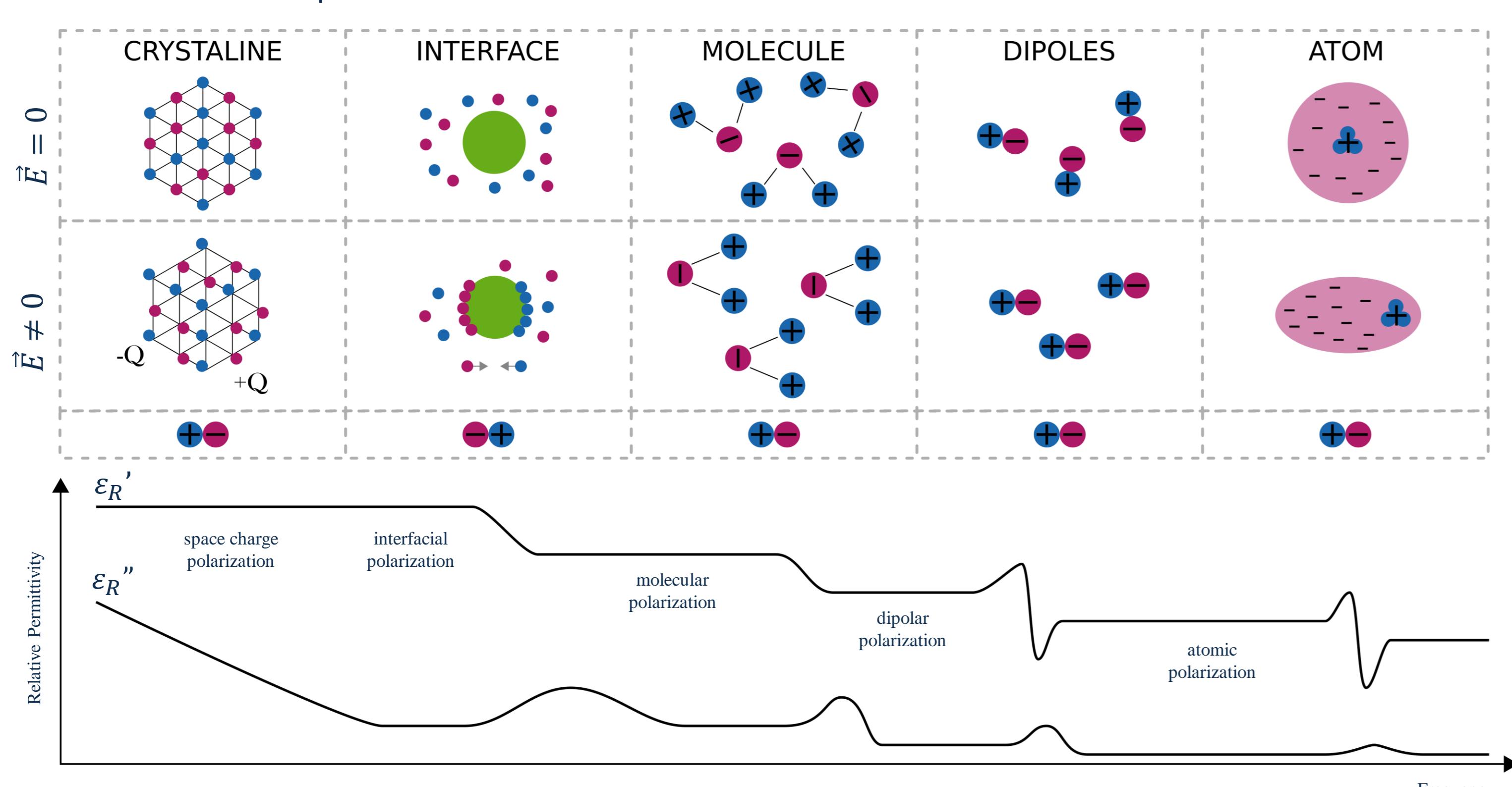


Figure 2 - Polarization Dispersion phenomena visually explained.

Source: Adapted from [8].

The second concept, intimately related to the concept of polarization is a special case of polarization when the dielectric medium is composed of two layers with different conductivities. This difference leads to the accumulation of charge carriers (ions) in the interface between layers (Fig. 3).

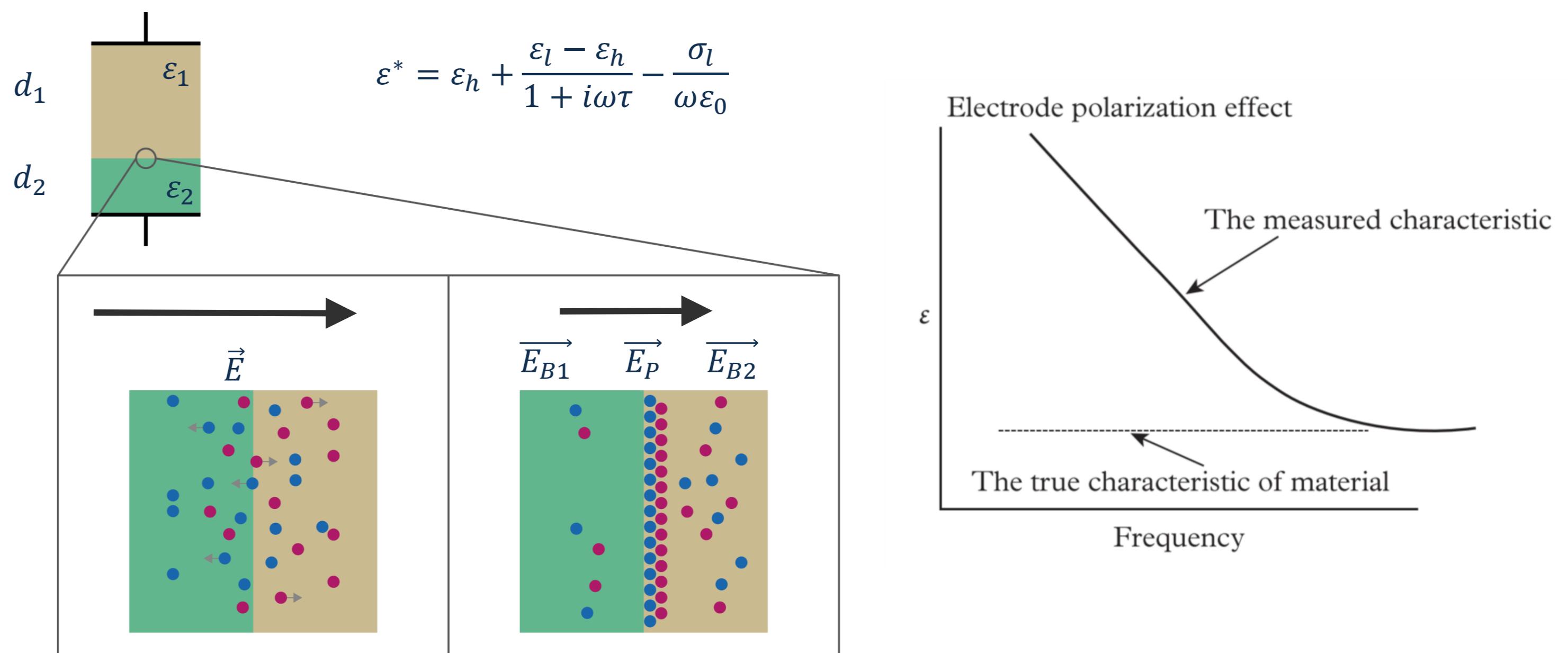


Figure 3 - Maxwell Wagner Phenomena.

Source: Adapted from [4][8]

When applied to biological or other highly conductive system containing ions, dielectric spectroscopy measurement techniques typically suffer due to the interfacial polarization. Due to the development of ionic double layers rapidly reduces the voltage drop applied in these layers, leading to the near-absence of the electric field within the bulk sample, specially at low frequencies [3-5]. Furthermore, as details in the electrode polarization depend on its microscopical structure and area, no simple correction technique has been widely accepted [8].

## RESULTS AND DISCUSSION

As has been presented, relative permittivity values are dispersed along the frequency spectrum due to the oscillatory behavior of matter when interacting with EM fields. As has been uncovered by Höber, EM waves propagate and, most importantly, are able to penetrate and interact with different cellular structures based on its frequency. Further development of the dispersive nature of biological cultures was led by Herman P. Schwan [6], in which the first truly broadband study of biological tissues was done. The investigation of propagation within cell cultures in frequencies ranging from 10Hz to 35GHz enabled the description of what are know as the  $\alpha$ -dispersion [1mHz-100kHz] (diffusion of ions in the electrical double layer surrounding charged particles),  $\beta$ -dispersion [100kHz-100MHz] (interfacial charging of cellular structures of tissues),  $\gamma$ -dispersion [15GHz-25GHz](rotational diffusion of water dipoles), and  $\delta$ -dispersion [100MHz-10GHz] (relaxation of water molecules bound to proteins and other surfaces in the tissue)

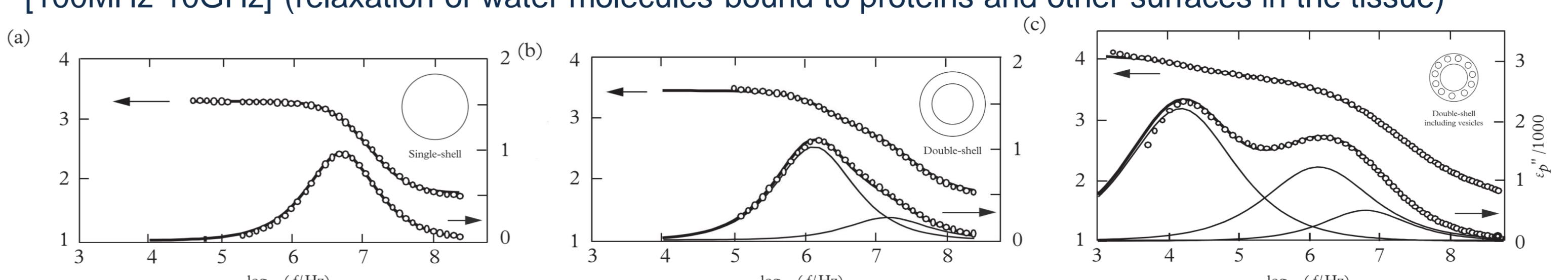


Figure 4 - Spherical shell dielectric models.

Source: Adapted from [8].

State of the art research of dielectric spectroscopy techniques strives for two major development routes. One approach that is chosen by many is to further development in Non-Linear Dielectric Spectroscopy (NLDS) methods, introduces by Woodward and Keil [7]. In contrast to the standard DS, NLDS used the relationship between voltages and currents at the electrode to determine the metabolic state of cell suspensions and in particular common brewer or bakers yeast cell suspensions. Although the technique is very promising to monitor metabolic changes of cell cultures over wide spans of time there are significant concerns with interfacial polarization phenomena, which mask out the effective  $\alpha$ -dispersion at low frequencies. No development of NDLS has been found to be done at higher frequencies, only at Hz and sub-Hz ranges.

In contrast, the other approach that has been taken by a few research groups is the development of standard DS at much higher frequencies. Although both narrow band and broadband techniques have been recently developed for DS in milli/microliter scales, much effort in the biological community has been aimed towards wide spans of frequencies. Oscillations below 10MHz reveal information about cell membrane resistance, capacitance and cytoplasmic conductivity, however investigation of subcellular components, such as vacuoles can only be performed at much higher frequencies [2] (100MHz-10GHz)

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