Dielectric Characterisation of Lipid Droplet Suspensions Using the Small Perturbation Technique

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Abstract. This work proposes a novel approach to differentiate biological cells based upon the total concentration of lipids. Lipid accumulation within cells is significant as it serves as a marker pertaining to the metabolism and oncologic state of the cell and organism. This is accomplished through dielectric characterisation of the sample. This chapter presents a preliminary proof of concept experiment using vegetable oils and cell culture media to model lipid droplets in biological cells. The experiment indicated that solutions of numerous different lipid suspensions at different concentrations can be differentiated based upon the dielectric characteristics of the sample. The dielectric constant of vegetable oils was calculated to be between 2.9 and 3.1. The dielectric constant of the suspensions reached up to 27 at a concentration of 0.5% (v/v).

Keywords: Cavity, dielectric spectroscopy, microwave, sensor, small perturbation, triacylglycerol.

1 Introduction

The incidence of malignant neoplasms has continued to rise for the past three decades and significantly contributes to population morbidity and mortality. Neoplasms occur when cell proliferation exceeds cell apoptosis (naturally initiated cell death) which results in the formation of a tumour or increased blood cell volume depending on the origin of the cancerous cell. Tumours can be difficult to identify in the early phase due to being asymptomatic with noticeable symptoms not arising until later development [1]. The earlier a neoplasm is identified the less invasive, simpler and cheaper treatments become resulting in increased patient comfort and prognosis [2-4]. Currently there are no quantitative or qualitative point-of-care diagnostic assays available to indicate neoplasmic growth. This

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paper proposes a novel method for assessing the lipid concentration of biological cells and a proof of concept experiment.

Under normal circumstances, most cells store small amounts of triacylglycerols (TAGs) in organelles called lipid droplets (lipid bodies, adiposomes). These are used to synthesise lipid membranes, other molecules of the lipid family and as a source of chemical energy and finally to synthesise signalling molecules [5, 6]. However, due to cellular mutations and/or abnormal cell to cell signalling, the metabolism of the cell is altered resulting in TAGs being accumulated in such lipid droplets [7, 8]. Depending on the genetic mutations that lead to neoplasm, a lipid droplet can constitute a significant sum of cell volume. Hence, the cell concentration of TAG is an excellent indicator or biomarker of cell metabolism and proliferative state.

The current methods for assessing the lipid profile of a cell or sample are lengthy, require specialised facilities and are subject to calculation induced error. The assay consists of hydrolysed fatty acids from the glycerol back bone employing lipase enzymes. The resulting glycerol is then quantified using colorimetric assays whereby an enzyme, usually glycerol kinase or glycerol triphosphate oxidase, reacts with the molecule producing hydrogen peroxide. This combined with a peroxidise results in a measurable colour change [9]. However, the drawbacks of this method are the inclusion of any free glycerol molecules already present and the patient must fast for up to 12 hours before the test [10]. It is currently employed as a marker of cardiovascular risk and metabolic diseases. Low density lipids (LDLs) and very low density lipids (VLDLs) can be calculated as a result of this and other tests. This test is currently the "gold standard" used in laboratories around the world and therefore is the benchmark to which the product of this research will be compared.

2 Characterisation Using Microwaves

Unlike chemical and fluorescent assessment methods of cellular lipid content, the dielectric characteristics of a cell may be used as a non-destructive indication of lipid accumulation. Biological samples, when exposed to radio and microwave frequencies of electromagnetic radiation, store an amount of electrical energy through an interaction with the molecular and structural properties of the sample [11]. The ability of the sample to store energy is described as the *permittivity*. Generally, the permittivity of a biological cell sample decreases in a series of stages known as dispersions: this phenomena is illustrated in Figure 1. Each step reflects the relaxation of a polarisation process.

Dielectric characterisation of cellular material is dependent upon polarisation processes that occur across a frequency range of Hz up to lower THz frequencies. Biological cells exhibit a characteristic decrease in permittivity as a function of increasing frequency known as α, β, δ and γ dispersions, details of which are noted briefly below.

- α dispersion is related to the adjacent flow of ions across the cell surface.

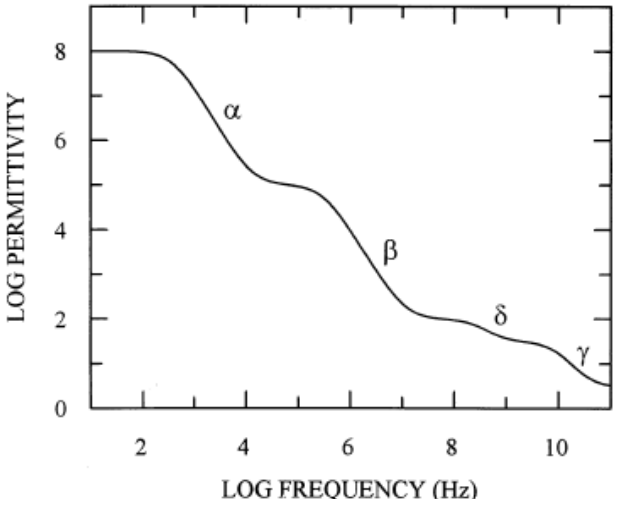


Fig. 1. Generalised dispersions of real permittivity associated with biological material

- β dispersion (also known as interfacial or Maxwell-Wagner interaction) is associated with the build-up of ions at the cell surface leading to polarisation across cell membranes.
- δ and γ dispersions are associated with molecular and sub-molecular rotations, predominantly bound and unbound water.

Neoplasmic processes alter biological characteristics, such as morphology and molecular composition consequently shifting the frequencies at which dispersions occur. Therefore, at a specific frequency the amount of energy stored will differ between normal and neoplasmic biological material. Many different dielectric spectroscopy techniques have been developed in an array of cancer diagnostic applications. The approach varies dependent on the site and type of cancer from characterising isolated individual cells to non-invasive in-vivo imaging techniques [12-14].

Dielectric spectroscopy may quantify permittivity of the material in the complex form. The complex permittivity is composed of the real and imaginary permittivity represented by the equation (1) where ε is the complex permittivity, ω is the angular frequency (ω = 2πf), σ the conductivity and j represents the imaginary unit (j = $\sqrt{-1}$) [15, 16].

$$\boldsymbol{\varepsilon}(\omega) = \varepsilon'(\omega) - j\varepsilon''(\omega) = \varepsilon_r + \frac{\boldsymbol{\sigma}}{j\omega} \tag{1}$$

$$\boldsymbol{\varepsilon_r} = \frac{\boldsymbol{\varepsilon}}{\varepsilon_0} = \frac{\varepsilon'(\omega) - j\varepsilon''(\omega)}{\varepsilon_0} = \varepsilon_r' - j\varepsilon_r'' \tag{2}$$

The permittivity of the sample under test is more commonly quantified as the relative permittivity whereby the permittivity of the material is calculated relative to

the permittivity of free space as in (2), where ε_r is the relative permittivity, ε0 is the permittivity of free space which is equal to 8.8541878×10^{-12} F/m , ε_r is the real part of the relative permittivity and ε_r is the imaginary part of the relative permittivity.

3 Experimental Methodology

The following experiment provides a simple preliminary proof of concept trial to determine if different conformations of vegetable oil and different concentrations of lipid droplets can be differentiated based on their dielectric properties at microwave frequencies. The oil suspensions serve as a cellular lipid droplet model whereby TAG is suspended in an ionic media similar to a lipid droplet within a neoplasmic cell. This method serves to validate the approach and technique to be implemented when assessing biological cells. The dielectric characteristics are calculated using the small perturbation method whereby a sample introduced to a resonating cavity shifts the frequency of resonant modes and decreases the quality factor dependant on the dielectric characteristics of the sample under test [17-19].

Samples of 100% grapeseed, groundnut, olive and sunflower oil (n=5) were prepared in sample tubes ready for dielectric measurement. Secondly, olive oil was suspended in YEPD media to yield concentrations of 50 to 0.5 (% v/v) oil (n=5). YEPD was prepared to the following protocol: 1 % (w/v) of yeast extract, 2 % (w/v) of bacto-peptone and 2 % (w/v) of dextrose diluted in de-ionised water. Before dielectric measurement, the sample would be thoroughly agitated for 15 seconds until a sufficient suspension was achieved.

Measurements were performed using an Agilent Technologies (Hewlett Packard) 8720 ET Vector Network Analyser (VNA) as illustrated in Fig. 2. The instrument was set to generate a signal between 1.5-1.8 GHz over 1601 data points for 10 linear frequency sweeps and calculate the S_{21} (transmission) parameters. Measurements were made through a custom cylindrical resonating cavity, designed and constructed to resonate at approximately 1.75 GHz at mode TM_{010} (see Fig. 3).

$$\varepsilon' = \frac{V_c\,(f_c - f_s)}{2V_s\,f_s} + 1 \tag{3}$$

$$\varepsilon'' = \frac{V_c}{4V_s}\left(\frac{1}{Q_s} - \frac{1}{Q_c}\right) \tag{4}$$

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \tag{5}$$

The dielectric constant, dielectric loss and loss tangent of the sample were then calculated using equations (3-5) [19], where ε' and ε'' are the dielectric constant and dielectric loss respectively, f_c and f_s are the resonant frequencies of the empty cavity and loaded cavity respectively, V_s and V_c are the volumes of the sample and cavity respectively and Q_c and Q_s are the quality factors of the empty cavity and the loaded cavity respectively. Precise measurement and documentation of the frequency shift and Q-factor by the VNA and custom GUI software reduced measurement error.

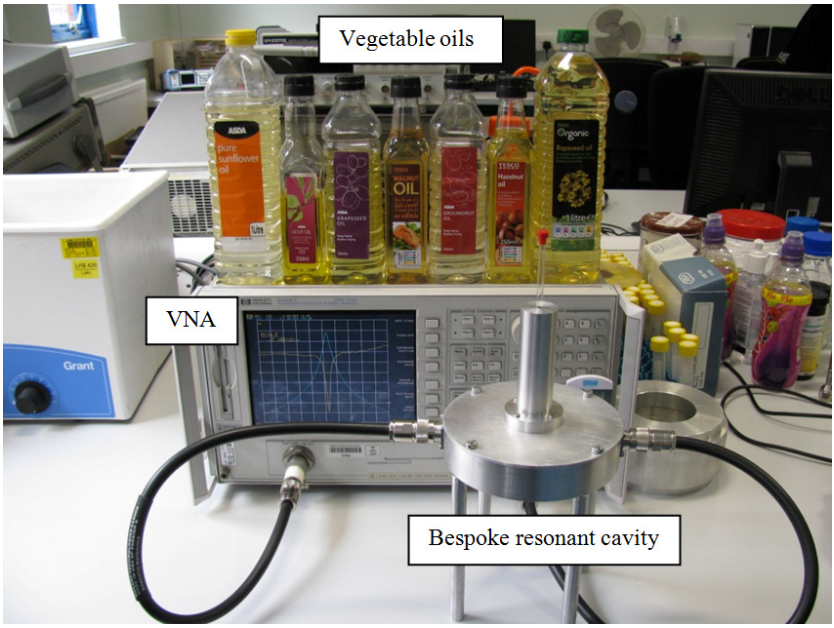
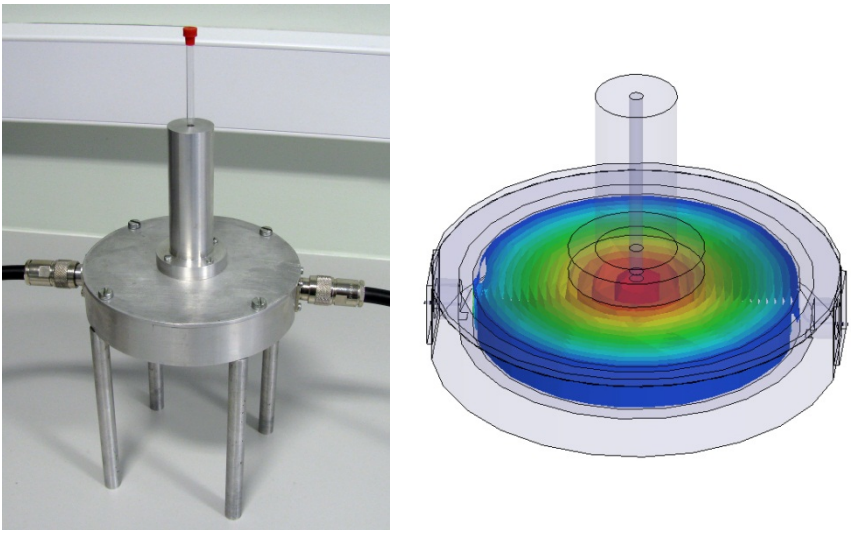


Fig. 2. Experimental setup, showing the range of oils used during experimentation, the microwave cavity and the VNA



(a) (b)

Fig. 3. (a) The microwave cavity used to assess the dielectric characteristics of the lipids, and (b) a simulation of the cavity showing TM_{010} mode, where the electric field is centred in the cavity at approximately 1.75 GHz

4 Results

Fig. 4 shows the relative dielectric constant plotted against the dielectric loss to represent the complex permittivity of vegetable oils at approximately 1.75 GHz. This is in general agreement with previous research that calculates the dielectric constant of vegetable oils to be 3.05 to 4.6 [20].

Table 1. Dielectric characteristics of vegetable oil

Veg. Oil	ε' (ω)	Std. Deviation	ε'' (ω)	Std. Deviation	tan δ	Std. Deviation
Grapeseed	3.014	0.0121	0.073	0.0005	0.024	0.0002
Groundnut	2.991	0.0119	0.066	0.0017	0.022	0.0007
Olive	3.004	0.0038	0.063	0.0005	0.021	0.0002
Sunflower	3.005	0.0115	0.069	0.0009	0.023	0.0003

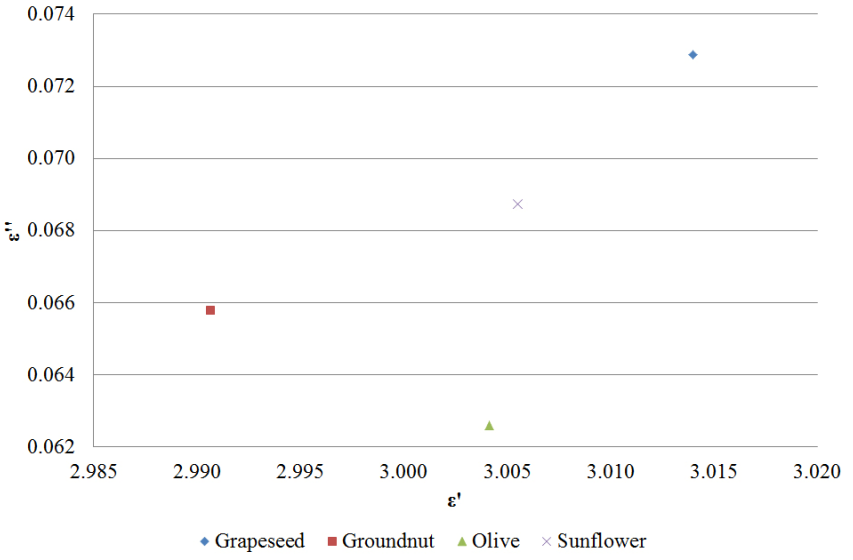


Fig. 4. Complex permittivity of Grapeseed, Groundnut, Olive and Sunflower Oil

Fig. 5 shows the relative dielectric constant and dielectric loss of olive oil suspensions. As the concentration of olive oil decreases within the suspension, both the dielectric constant and dielectric loss increase up to 27 and 2.5 respectively.

In Fig. 6 the dielectric loss was plotted against the relative dielectric constant representing the complex permittivity. As the concentration of lipid droplet increases and the YEPD media inversely decreases, the permittivity of the sample decreases. Detailed results are shown in Table 1 and 2.

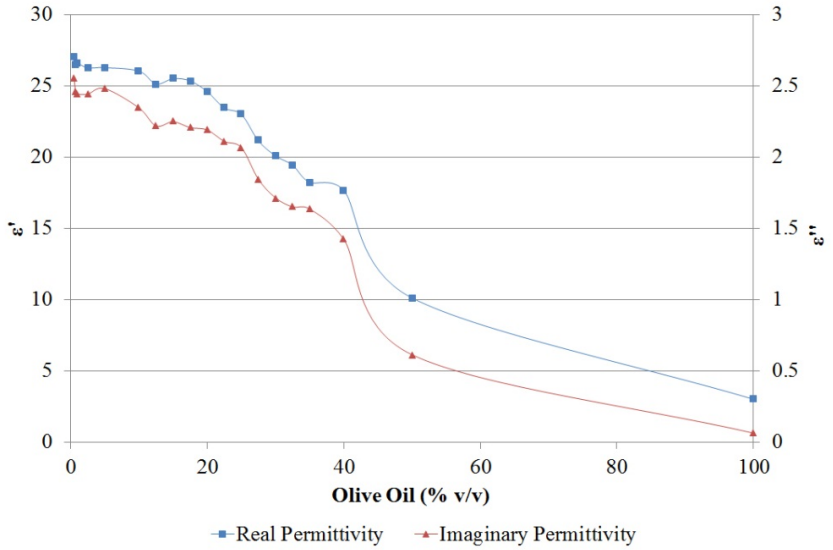


Fig. 5. Graph showing a decrease in relative dielectric constant and dielectric loss as the concentration of olive oil increases

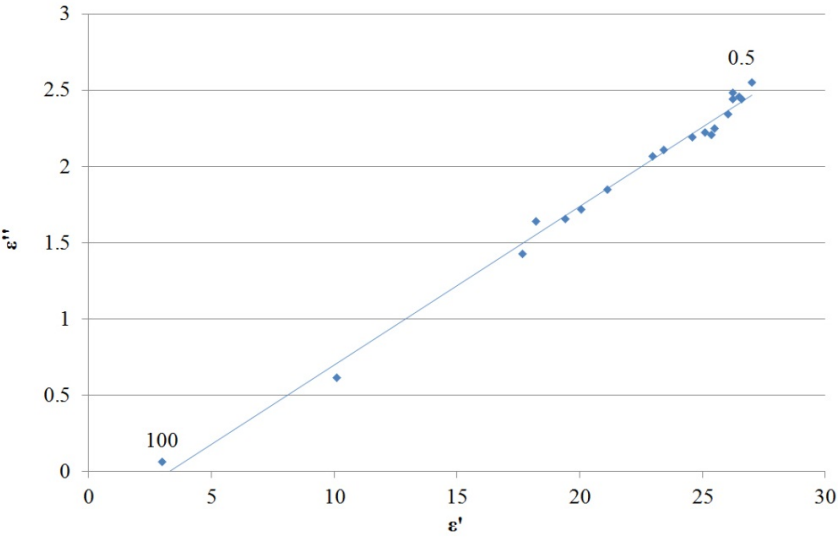


Fig. 6. Plot of relative dielectric constant against dielectric loss to represent complex permittivity. Each data point indicates a concentration of olive oil suspension with oil concentration decreasing as the complex permittivity increases

Table 2. Dielectric characteristics of lipid droplet samples

Olive Oil (% v/v)	ε' (ω)	Std. Deviation	ε'' (ω)	Std. Deviation	tan δ	Std. Deviation
100	3.004	0.0038	0.063	0.0005	0.021	0.0002
50	10.106	1.6102	0.612	0.1652	0.059	0.0054
40	17.658	1.5520	1.423	0.1675	0.080	0.0044
35	18.215	1.1339	1.635	0.0467	0.090	0.0031
32.5	19.423	1.0756	1.650	0.1395	0.085	0.0029
30	20.078	0.9285	1.714	0.1394	0.085	0.0032
27.5	21.138	0.0118	1.844	0.0017	0.087	0.0006
25	22.992	0.6427	2.066	0.0678	0.090	0.0020
22.5	23.434	0.9851	2.110	0.1027	0.090	0.0014
20	24.585	0.3151	2.190	0.0256	0.089	0.0016
17.5	25.336	0.4128	2.206	0.0606	0.087	0.0014
15	25.489	0.2968	2.247	0.0365	0.088	0.0005
12.5	25.118	0.2618	2.217	0.01400	0.088	0.0005
10	26.028	0.3046	2.345	0.0733	0.090	0.0034
5	26.269	0.2545	2.480	0.1030	0.094	0.0033
2.5	26.256	0.3081	2.441	0.0271	0.093	0.0018
1	26.588	0.4961	2.441	0.1034	0.093	0.0052
0.75	26.504	0.0118	2.453	0.0017	0.093	0.0006
0.5	27.024	0.3939	2.549	0.0343	0.094	0.0019

5 Discussion

This experiment has shown that differing conformations of TAG and different concentrations of lipid droplets can be distinguished based on the electromagnetic properties of the suspensions. The differing concentrations cause a frequency shift indicating a change in dielectric constant and losses of the samples at this frequency range. However, the molecular and structural composition, interactions between molecules, interfaces between substrates and the frequency employed must be considered as to the contribution to the dielectric qualities of the samples under test.

A range of vegetable oils were used to investigate how varying proportions of TAG species affected the dielectric characteristics. Groundnut oil, which is relatively low in mono-unsaturated TAGs (21%), has a relative dielectric constant of 2.991 while grapeseed oil with comparatively higher concentrations of mono-unsaturated TAGs (53%) has a dielectric constant of 3.014. This is in agreement

with literature that reports an increase in dielectric constant as the proportion of mono-unsaturated TAGs increases.

When the oil mixtures are agitated, the oil does not dissolve into the YEPD media. Instead the hydrophobic oil converges into lipid droplets resulting in a colloidal sample. At lower frequencies (MHz region) charge builds up at the interface of the separate phases known as the Maxwell-Wagner effect and is responsible for the β-dispersion. At the frequency of this experiment however it is likely that the major influence to relative complex permittivity is attributable to the organic and ionic content of the YEPD media. The media contains a combination of molecules necessary for yeast culture namely carbohydrates (sugars), amino acids (protein) and minerals (salt). Such molecules are well known to interact in aqueous solution, lowering the relaxation frequency of the water molecules into roughly the lower GHz frequency range [21]. This loss of polarisation is known as the δ dispersion attributable to the rotation of molecular side chains and bound water. Increasing the proportion of media increases the amount of material that can be polarised increasing the amount of energy that can be stored by the sample.

After agitation, as the sample is introduced to the cavity the lipid droplets begin to converge into larger droplets until the lipid begins to separate into a different phase resting on the YEPD media. This results in the lipid droplet surface area being lower than the newly agitated suspension. As this process occurred, a decrease in the perturbation frequency was noted signifying a decrease in permittivity. Therefore, it is probable that a change to the β dispersion, due to the decreasing lipid droplet surface area, may contribute to the complex permittivity of the sample at this frequency. Acquiring data periodically from agitation to phase separation could be carried out to investigate the consequence of a changing droplet surface area. Stabilisation of the lipid droplets using phospholipids would improve the homogeneity of the suspensions also improving measurement repeatability.

6 Conclusions

This research proposes a novel, non-invasive diagnostic approach for the detection of neoplasmic growth. The aim of the research has been to develop a microwave sensor and methodology to assess the lipid profile of yeast cultures. Experiments conducted so far have validated the applicability of the method for detecting lipid accumulation in biological cells using a lipid droplet model.

The technique may in the future be adapted for use with mammalian cell cultures and, finally, isolated cells from the body. The approach has the potential to be fast, safe and relatively inexpensive allowing a commercialised product to be easily rolled out across healthcare facilities.

As part of on-going work, a method has been devised to reliably insert a test sample into an EM field generated by a small microwave frequency sensor. The method utilises a platform with an engraved grid pattern into which a microtitre plate can be slotted. The microtitre plate can then be repositioned so each of the wells will be interacting with the sensor. The platform in Fig. 7 is designed for 24

well plates. The sensor mounting is composed of a recess cut into the platform. Realised sensors can be secured into the sensor mounting of the platform allowing the performance of different sensors to be evaluated under similar conditions. Further work may also consider the implication of environmental factors such as temperature and humidity which may influence sensor response.

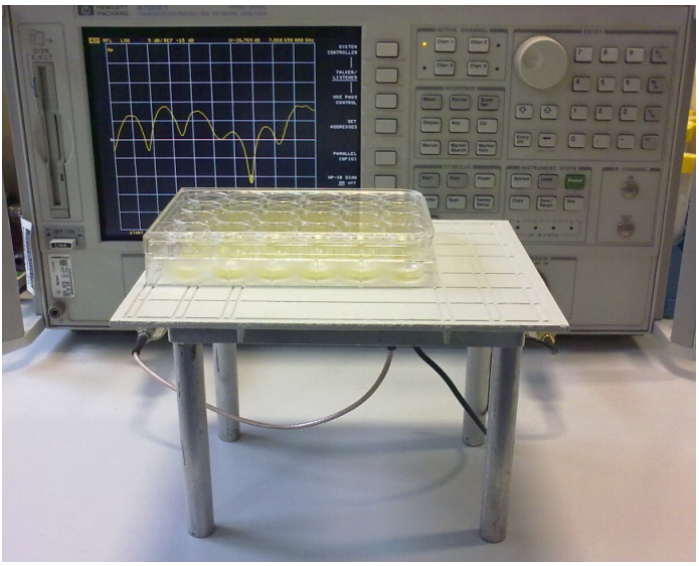


Fig. 7. On-going advancement of work to consider alternative format microwave sensors applicable for use with cell cultures presented in microtitre plates

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