High frequency fluidic and microfluidic sensors for contactless dielectric and in vitro cell culture measurement applications

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Abstract. There is a widespread need for highly-sensitive robust sensors that operate without direct contact to the fluid for analysis of fluids in bioprocess technology. Measuring the variation of dielectric properties (conductivity and permittivity) in the microwave frequency band can be used as an approach to investigate biological and chemical matter and processes such as, cell growth, cell metabolism and the concentration of large aqueous based molecules. In comparison to measurement at lower frequencies, DC conductivity ($\sigma$) effects on material properties ($\varepsilon$) can be neglected with increasing of the frequency. This presentation describes a high frequency sensor, which combines detection in macro- or microfluidic networks with quick and precise analysis. It is composed of a fluidic channel placed contactless between a micro-strip line waveguide combined with resonant properties.

1. Introduction
The use of flexibly constructed lab-on-a-chip diagnostics systems with many interchangeable modules will allow a cost-effective implementation of analytical functions across a wide application spectrum. Through the use of small microelectronic systems and miniaturised sensor modules, biological interaction mechanisms can be used to implement easy-to-use medical diagnostic systems, for process control in the food industry and for environmental monitoring. Microfluidic systems find various applications: (i) classical chemical/biochemical analysis, (ii) microreactors for chemical synthesis, (iii) drug development, (iv) diagnostics, (v) DNA amplification, (vi) cell biology, (vii) tissue engineering and many more. There have been a huge number of publications describing the application, construction and challenges of microfluidic systems in the last 15 years [1, 2].

Contactless measurement by capacitive coupling of the excitation signal avoids many of the electrode effects associated with direct electrode contact [3]. At RF frequencies, contactless electrodes operate within a very narrow band and have non-linear characteristics within the plateau phase of the $\beta$-dispersion and the influence of medium conductivity changes directly influences the measured capacitance. Contactless measurements at microwave frequencies determine the sum of polarizable contents. The integrity of the cell membrane and in turn the cell’s viability cannot be assessed at frequencies greater than 50 MHz. In any case, the growth characteristics of batch cell cultures can be ascertained by measuring the influence of metabolites on the permittivity and conductivity of the medium. These properties enable the development of miniaturized sensors based on high-frequency analysis for fluidic and microfluidic applications.
2. Materials and Methods

Microwave sensors measure the interaction of matter with high frequency electromagnetic electric fields. The interaction of microwave radiation with polar materials, for instance, water, depends on how the polarized molecules orient themselves to the applied field. If the polarity of the field reverses, the molecules reorient themselves. The phase difference of the molecules with regard to the applied high-frequency electrical field is described macroscopically by the complex relative permittivity. The relative permittivity expresses how much greater the capacitance of a capacitor would be if air ($\varepsilon_r = 1$) was replaced by a dielectric material of identical thickness. The real part of the complex relative permittivity $\varepsilon_r'$ depends on the polarisability of the material. The imaginary part of the complex relative permittivity ($\varepsilon_r''$) depends on the dielectric losses through absorption of energy during the reversal of polarity and on Ohmic losses. The frequency-dependent conductivity $\sigma'$ is related to the imaginary part of the complex relative permittivity. The direct current (DC) conductivity $\sigma_{DC}$ must be taken into account when calculating the permittivity of higher conductivity media, such as seawater [4] or culture medium. Above 100 MHz, the influence of the moisture content and conductivity (ionic strength) of the object under test can be measured by separating the complex permittivity into its real and imaginary parts (1):

$$\varepsilon_r(\omega) = \varepsilon'_r(\omega) - j\varepsilon''_r(\omega) - j\frac{\sigma_{DC}}{\varepsilon_0 \omega}$$  \hspace{1cm} (1)

Measurements with microwave sensors are particularly well suited for measurement application in non-destructive testing because microwaves can penetrate deep into the material without any direct contact and can even be transmitted through the material. For example the permittivity of water differs strongly from that of other liquids and aqueous mixtures ($\varepsilon_{H2O} \approx 80.5$, alcohol with $\varepsilon_{C2H5OH} \approx 24.1$, cellulose with $\varepsilon_{Cellulose} \approx 3.05$ at 1 GHz frequency and 25°C temperature), and this property highlights the great potential microwave sensors have for precise and fast applications in medicine, biotechnology and food technology. A variety of applicator designs have been described with regard to solving the basic electromagnetics. Concerning the coupling of applicator and object under test, the general types of applicators are: a) strip line applicators, b) stray field applicators, c) radiation field applicators, d) resonators and e) hybrid forms of the previously listed applicators. It is suggested that readers refer to these resources for a complete description of the relevant theory and practical approaches regarding the interaction of microwaves with dielectric materials [5, 6].

The compact sensor consists of a flow-through chamber; tubing can be reproducibly placed between strip-line electrodes that act as the sensor element (Figure 1). The tubing can be easily placed in the sensor and replacement is uncomplicated. Media can be pumped through the tubing so that the variation of the material under test is measured by the MW-Sensor. The strip-line waveguide is made from two copper cantilevers of 36 mm length and 3 mm width. The cantilevers were separated from each other by 3 mm. The strip-line waveguide was placed inside an aluminium housing (51 x 26 mm) and connected directly to the soldering terminals of an SMA connector.

Fig. 2 shows schematically how the frequency response of a resonator can be described by the width at half maximum, $W$, and the area, $A$, where the reflection coefficient ($S_{11}$ expressed in dB) is presented as a function of frequency for liquids with varying water content. These electrical properties are the basis of numerous calculations for the determination of the complex permittivity of mixtures or as a calibration function for determining the composition of material compounds. Sometimes the exact estimation of the resonant frequency and the width at half maximum was only possible at reduced accuracy. For this reason, equation 2 was developed, to improve the calculation of the area underneath a resonant curve (in this equation SI is a reflection index).

$$S_{11} = \frac{A}{f_0 - f_r}$$  \hspace{1cm} (2)

Further detail of this measurement set up (sensor design and data processing) can be found in [7].
3. Experiment and Results
The performance of the sensor was tested using several liquids with well-known dielectric and conductive properties: air, ethanol, deionized water, salt solution with different ionic concentrations and mixtures (Fig: 3, Fig 4). A special application was the determination of the ethanol concentration from a condensate during ethanol fermentation (Fig. 5) and the growth of s. cerevisiae in growth medium BM001B (SY-LAB) inoculated with 2.6x10⁵ cells per ml. Measurements where performed in an incubator at a controlled temperature of 25°C. The complex reflection coefficient S₁₁ was measured with a network analyzer (Anritsu MS MS4644A).
Figure 5. Growth of S. cerevisiae microorganisms in a stirred culture medium as a batch, fermentation time 5 h, a) shows the spectra and b) shows the time duration of the exponential grown phase

4. Conclusion and Future Work
The contactless nature of these sensors enables the cost-effective production of microwave sensors that can avoid sample contamination. Numerical simulation with high-frequency full wave electromagnetic software is necessary for optimization of the development of these sensors. Reproducible measurements require high precision in the mechanical production of the geometry of each sensor: obviously microfabrication offers such specifications. The next step will be the determination of the variation of conductivity and dielectric properties of a cell culture in a droplet application (volume >100 nl) in a segmented flow system. The number of cells, consumption of nutrients and cell metabolism directly influence the passive dielectric properties.

Together with reasonably priced high frequency electronic circuits (for example mini-circuits delivered high frequency synthesizers and Analog Deveces hole RF Gain-Phase Detector with bandwidths up to 2.7 GHz and the design and manufacturing of a scalar MW Device is also much cheaper than a laboratory system) this techniques opens up the possibility of automation simplification and miniaturization of routine application and in a wide variety of sample pretreatments.

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