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## Sensor strategies for microorganism detection – from physical principles to imprinting procedures

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**Abstract** Detecting cells and microorganisms in different matrices is becoming an increasingly important task in a variety of fields including bioprocess control, food technology, health care, and environmental analysis. In this review, fast on-line detection methods for this purpose are presented including different recognition and transducer strategies.

**Keywords** Microorganism detection · Sensor · QCM · SAW · SPR · imprinting

### Introduction

Although healthcare and medicine have made fundamental and outstanding progress in curing infectious disease, microorganism contamination is still a substantial threat to man. Therefore, detection methods for infection and microbial toxins are essential in fields like food industry, water supply or hospital hygiene. However, they can also become general public concerns regarding, e.g., the recent demand for tracing anthrax spores. Well established chemical and biological analytical methods rely on inoculating a culture medium with the sample and breeding the microorganisms followed by counting the colonies present after a defined amount of time or on polymerase chain reactions (PCR) synthesizing parts of the contaminant genotype followed by a suitable chemical analysis, e.g., electrophoresis, which can also be used for separating entire biological species [1]. All these methods are highly suitable for the task and yield the desired detection limits, the analysis time, however, is usually too long for rapid determinations, as, e.g., cell cultivations on media can last up to several days. During the last decade a lot of effort has been made to overcome this problem, this is also a re-

sult of the progress made in the field of chemical and biochemical sensing including a variety of new detection techniques and developments in device design. Of course, miniaturization of established analytical methods is one possibility, an extensive review on this topic can be found in Ref. [2], where different detection and assay types are introduced and discussed according to their specific advantages and drawbacks. In contrast to that, our review will cover chemical sensor systems and omit the lab on a chip approaches [3], as the resulting devices do not count as sensors in the classical sense.

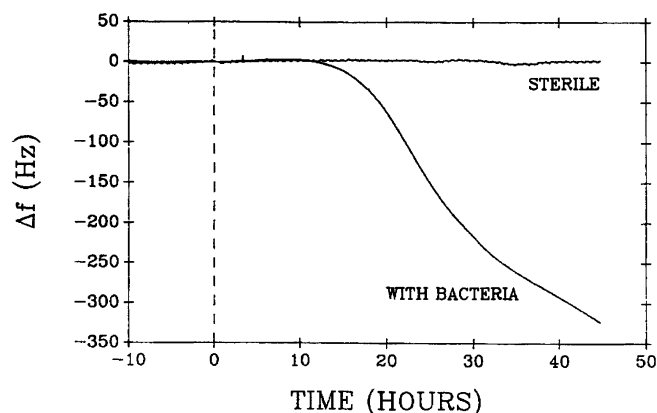
Some sensor systems (mainly so-called electronic noses) are already commercially available and mainly used for analyzing gas mixtures. This strategy is used for indirectly determining microorganisms leading to the spoilage of milk [4] by monitoring their metabolism products. Therefore, in this case, not the contaminating cells are directly measured but the headspace gas over the milk is characterized by a sensor array consisting of 14 different conducting polymers. Data evaluation by the means of discriminant function analysis (DFA) and principal component analysis (PCA) allows not only for discriminating between spoiled and unspoiled milk but also yields information about the type of contaminant microbe as each microorganism induces a specific response patterns of the gas sensors.

A different approach to the sensing of bioactive materials, that will not be covered within this report, is the design of microbial biosensors, where viable and/or dead cells are deposited on a transducer and serve as selective “material”, an extensive review on this topic can be found in Ref. [5], a recent report of a multiparametric neurosensor microchip in Ref. [6]. In the following sections different type of bio- and chemosensors for detecting cells in a variety of samples will be introduced, the systems are sorted according to their transducer principle.

### Acoustic sensors

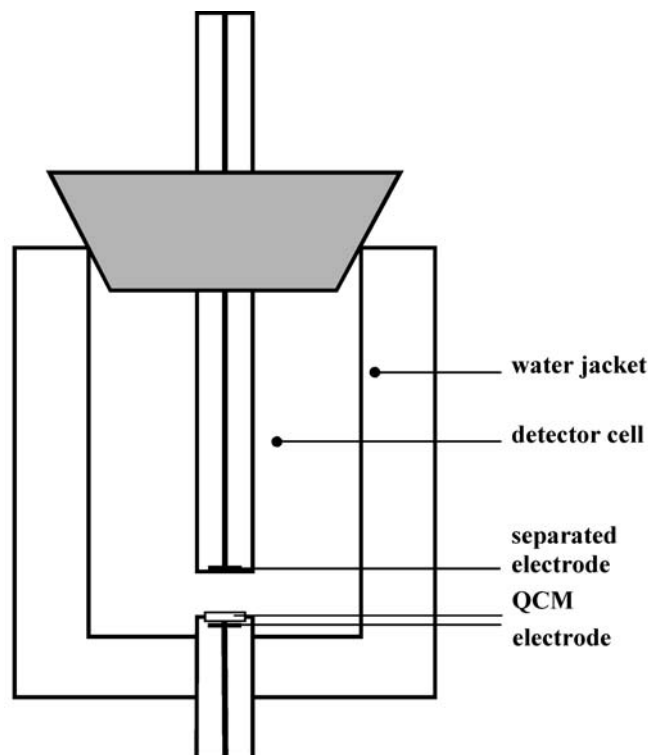
Acoustic sensors have already found widespread application in chemical sensing, the most prominent devices used

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**Fig. 1** Effect of *Pseudomonas aeruginosa* film formation on a 5 MHz-QCM (Ref. 9)

are the quartz crystal microbalance (QCM) and the surface acoustic wave resonator (SAW). These types of transducer rely on piezoelectric materials and convert a mechanical signal, namely a change in mass, into a shift of resonant frequency. Two excellent reviews of biosensing with acoustic resonators can be found, one dealing mainly with QCM [7], the second article [8] additionally covers SAW sensors. Both articles also present the reader with an introduction into the functional principles of mass-sensitive sensing and give a short summary of the theoretical background. A wide range of biosensing including mainly the detection of biopolymers but also of microorganisms by a variety of methods, such as using uncoated devices or immunochemical detection is missed. One of the earliest applications of uncoated QCM resonators for the detection of biological samples was given by Nivens and coworkers [9]. They reported the on-line monitoring of *Pseudomonas cepacia*, which is a common contaminant even in ultrapure water systems, by the means of a 5-MHz QCM in an oscillator circuit. For measurements, two QCM devices are placed in a flow-through cell and each driven by an oscillator, through one of the cells culture medium is passed whereas the quartz in the other one is exposed to *P. cepacia*. Bacterial colony formation on the QCM electrodes leads to a decrease in resonance frequency correlated with the continuing growth of the film (see also Fig. 1). The method is validated by a classical cell-count procedure leading to a calibration function of the sensor and a lower detection limit of  $3 \times 10^5$  cells/cm<sup>2</sup>. Helle et al. [10] used 10-MHz QCM resonators to follow biofilm growth of different microorganisms with variable nutrient concentration and flow rates on electrode surfaces. The device is mounted on the top of a steel pipe and the culture medium with the growing cells fed by a peristaltic pump. Using the device admittance rather than just the resonance frequency is the main improvement suggested in this paper. Analytes include a variety of different biofilm-forming species, such as *Staphylococcus warneri*, *Bacillus coagulans*, *Burkholderia cepacia* isolated from paper machines. Results indicate that biofilm formation is independent of whether polished or rough electrode sur-



**Fig. 2** Separated electrode piezoelectric resonator (Ref. 11)

faces are used, however, it is highly dependent on parameters such as nutrient conditions, pressure fluctuations (when using a laminar flow system) and, of course, temperature. Finally, two other points have to be taken into account, namely the facts that biofilms are not necessarily firmly attached to the electrode surface and film thicknesses change during growth. A different strategy for cell sensing is given by Yao and coworkers [11], who modified the QCM device. They propose a so-called separated electrode piezoelectric quartz sensor shown in Fig. 2, where only one face of the AT-cut quartz contains an electrode. The other one is realized by a contacted platinum disk placed 16 mm away from the QCM surface. Thus the resulting resonance frequency strongly depends on the impedance of the liquid medium between the quartz and the counter electrode. The authors use this setup to determine *E. coli* by inoculating a culture medium and following the sensor signal with time. Above a certain threshold value, the bacterial concentration reaches a detectable value leading to a positive frequency shift in the range of some kHz. It has been found that the so-called frequency detection time (FTD), i.e. the time ranging from inoculation to the onset of the frequency shift, is strongly depending on the initial concentration of *E. coli*. Bacteria can be detected in initial concentrations between  $10^0$  to  $10^6$  cells ml<sup>-1</sup>, detection time is less than ten hours, which is substantially faster than the classical cultivation methods.

Viable or dead cells, however, do not necessarily have the same impact on a QCM resonator as small molecules, thus do not fulfil the premise of an ideally rigid mass layer

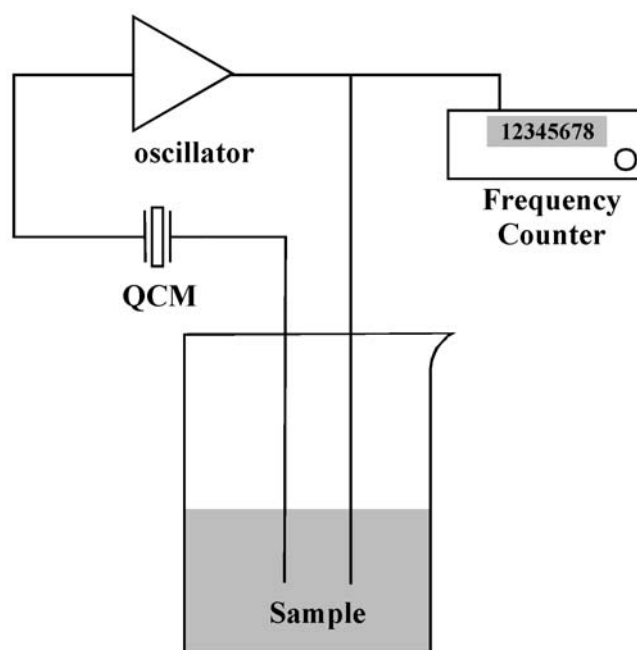
on the electrodes. Therefore, frequency shifts may not contain the entire information about cell adhesion, as only partly adsorbed microorganisms retain a certain mobility on the surface leading to viscoelastic dissipative effects. Hermansson et al. [12] give a profound study of these phenomena by following both the frequency shifts, i.e., the mass change on the surface, as well as the dissipation changes, i.e., variations in viscoelastic interactions, due to the adsorption of bacteria. By using different mutant strains of *E. coli* (fimbriated and non-fimbriated ones) parameters influencing the interaction between the cell wall and the device surface can be assessed. Nonfimbriated cells for example are bound much better to the (relatively hydrophobic) gold electrode with increasing ionic strength. Determining  $dD/df$  allows the calculation the dissipative energy per contact point, leading to a measure for the type and strength of interaction between different *E. coli* strains and the surface. Furthermore, both signals increase with time indicating growing contact areas and viscoelastic losses per cell.

Several efforts have been made to improve the selectivity of the devices for discriminating between different microorganisms or specifically detecting only one species. The most straightforward way to achieve this is the direct immobilization of antibodies on the surface of a suitable transducer. A very early work utilizing this strategy was published by Karube and coworkers [13], who reported a fast and comparably easy detection method for *Candida albicans*, a bacterium ubiquitously present in the human population. The otherwise harmless microorganism can be hazardous for persons with a stressed immune system, therefore it plays an important role in clinical analysis. Anti-*Candida* antibodies are anchored on the electrodes of 9-MHz QCM devices with palladium electrodes by a multi-step procedure: anodic oxidation of the electrode materials followed by condensation of the resulting reactive groups with  $\gamma$ -amino-propyl-thriethoxysilane, coupling glutaraldehyde on it and using the aldehyde functionality to anchor an Anti-*Candida* antibody. *C. albicans* is detected by a dip-and-dry procedure, where the sensor is submersed in the sample, left there for 30 minutes, rinsed and dried. Before and after this procedure, the resonance frequency is determined leading to the sensor signal. In such a manner, microbe concentrations between  $1 \times 10^6$  cells  $\text{ml}^{-1}$  and  $5 \times 10^8$  cells  $\text{ml}^{-1}$  can be detected much faster than via classical immunoassays. Testing with *Saccharomyces cerevisiae* reveal no cross-sensitivity of the sensor in this concentration range. The same strategy was used for the detection of erythrocytes in clean solution or whole blood [14]. In this case 10-MHz QCMs with gold electrodes are used in a dip-and-dry procedure, thermal fluctuations and unspecific interactions are corrected for by an uncoated quartz. Anti-glycophorin A is used as antibody towards the erythrocytes, after testing different immobilization methods the authors suggest the use of polyethyleneimine and glucaraldehyde for this procedure. When exposed to a solution of purified human erythrocytes or to whole blood the sensor shows a linear response characteristic within 10 minutes in a concentration span

between  $1 \times 10^3$  cells  $\text{ml}^{-1}$  and  $5 \times 10^4$  cells  $\text{ml}^{-1}$ , above this range the sensor signal is saturated. Additionally, the crystal-based sensor with the optimized anchoring layer can be re-used five times or 15 days, respectively.

Deng et al. [15] proposed a detection method for *Salmonella typhimurium* using a QCM oscillator, however, in this case the resonator device, however, is not directly interacting with the sample liquid but the quartz is in serial connection with the actual sensing chamber. As seen in Fig. 3, this contains two platinum electrodes and thus forms a cell for impedance measurements. Therefore, the resonance frequency of the oscillator circuit is highly influenced by impedance changes in the medium between the two platinum electrodes (the resonance frequency is “pulled” by a variable serial resistor). For detection, a sterile agar or culture medium is placed into the reaction chamber between the electrodes (in case of a gel they are immersed to about 10 mm) and a solution containing *S. typhimurium* is added. The chamber is incubated at  $37^\circ\text{C}$  and the frequency monitored as a function of time. After a defined period the QCM signal changes as a result of the growing bacteria that lead to change in solution (or gel) impedance. Once again, the frequency detection time is monitored as a function of the initial microorganism content in the sample, the sensor characteristic obtained shows the detection of *S. typhimurium* in a concentration range between  $10^2$ – $3.6 \times 10^6$  cells  $\text{ml}^{-1}$ .

A similar apparatus for the detection of *E. coli* was proposed by a group led by Wei [16]. However, the strategy for cell detection in this case does not rely on the immobilization of an antibody but makes use of *Tachypleus ambeocyte* lysate. It has been found that this coagulates in the presence of enterotoxins and coliform bacteria contain



**Fig. 3** Block diagram of the measuring apparatus with external QCM for *E. coli* detection (Ref. 15)

a huge amount of such compounds in their outer cell membrane. For sensing, a 9-MHz QCM with silver electrodes is immersed into a solution containing both the lysate and the sample. The measuring chamber is kept at 37 °C and the frequency response of the quartz is monitored as a function of time. Gelation occurs within less than an hour, which can be easily detected by the quartz as a result of the changing viscoelastic behavior of the liquid around the quartz. Plotting the frequency shifts against cell concentration yields the sensor curve that indicates a detection range of  $2.1 \times 10^4$  to  $2.1 \times 10^8$  cells  $\text{ml}^{-1}$ .

Howe and Harding [17] propose a different strategy for the detection of bacteria by reversing the usual detection protocol as they immobilize the microorganism rather than the antibody. The main advantage of this system is the better stability of the signals: as cells are much larger and heavier than any antibody and additionally have a curved surface, ideal contact with the proteins on the electrodes cannot be achieved, the bacteria can even sometimes remove the proteins from the surface. For unmodified quartz surfaces, bacteria are ideally deposited at pH=4, under these conditions they are tightly bound to the surface, when the microorganism suspension is deposited on a SAW device (Love-wave resonator with 124 MHz) and exposed to the antibody. The frequency shift recorded is a measure of the number of cells present on the surface of the device, which, of course, depends on the initial bacteria concentration in the coating liquid, in this manner, *E. coli* and *Legionella* can be detected down to  $4 \times 10^5$  cells  $\text{ml}^{-1}$ , by using two-electrode resonators this can even be done simultaneously.

Antibody-coated QCM sensors have also been reported for the detection of viruses. Gajendragad et al. [18] propose a dip-and-dry sensor system for determining foot-and-mouth disease in tissue samples. Virus-specific antibodies are produced in rabbits and guinea pigs and immobilized on the silver electrodes of a 6-MHz QCM by directly pipetting the antibody solution onto the device and evaporating the solvent. For the determination of an infection, 1  $\mu\text{l}$  of a clinical sample is pipetted onto the quartz, after incubation the device is washed and dried. When the resulting frequency shift exceeds a certain threshold value (2.5 Hz), the test result is positive. The shelf life of the sensors has been determined to be 18 weeks, it can be reused eight times and gives reliable results within a range of one hour. Another work dealing with the determination of viral infections directly in natural samples has been published by Wong and co-workers [19], who determined the infection of orchids with cymbidium mosaic potexvirus and the odontoglossum ringspot tobamovirus. The authors discuss a variety of different antibody immobilization methods, modifying the surface with 3,3'-thiopropionic acid and incubating this with *N*-hydroxysuccinimide yields the best results. With this method the viruses are successfully detected in crude plant saps, as can be seen by the sensitivity pattern shown in Fig. 4. Sensors produced this way can be stored for six months without losing their selectivity and can be re-used five times thus exhibiting a reasonable shelf life for a commercial application.

Biological recognition materials show excellent sensitivity towards cells, their main drawback, however, is the limited stability of the resulting sensor layers. A possible method of overcoming this limitation is the use of artificial antibodies such as molecularly imprinted polymers [20, 21, 22], where an image of the analyte is produced in a matrix by templating procedures and self organization. For microorganisms usually surface imprinting procedures are used, early examples for bacteria-functionalized polymers were developed by the Vulfson group [23, 24]:

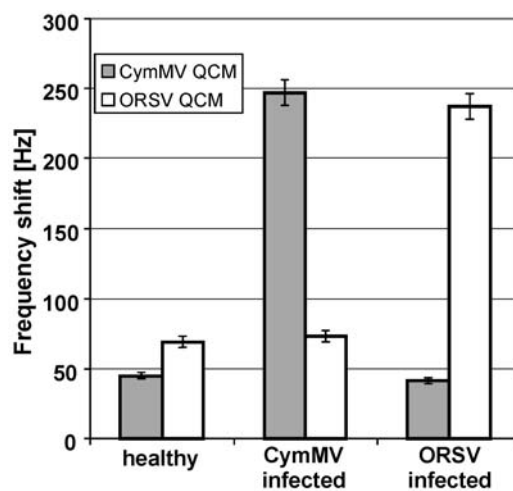


Fig. 4 Sensitivity pattern of plant virus biosensor (Ref. 19)

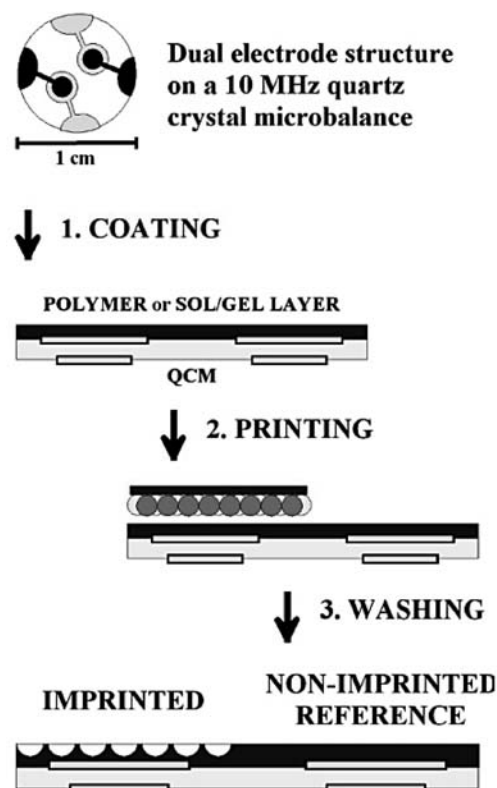
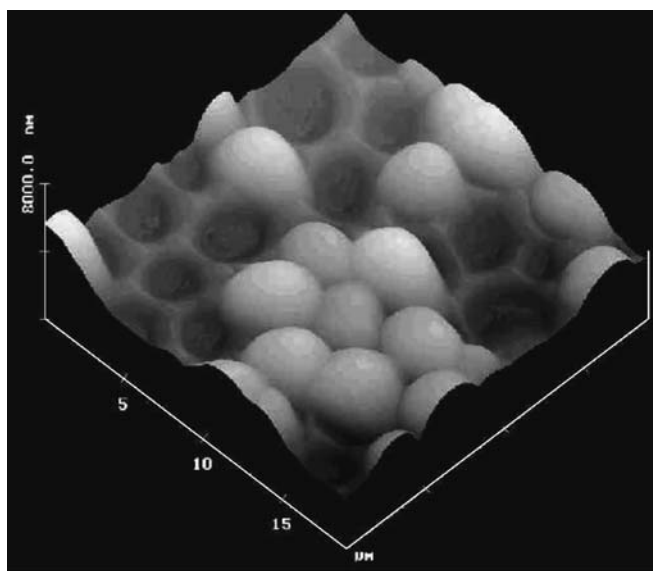
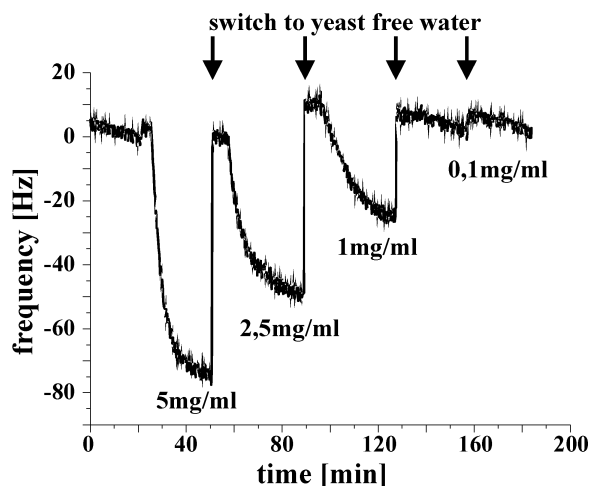


Fig. 5 Cell imprinting procedure

For the development of sensor systems, e.g., yeasts are pressed into a pre-polymerized reaction mixture of an organic polymer[25] or a sol-gel glass (Fig. 5). After polymerization has finished, the cells are washed from the surface leaving behind a honeycomb structure of cavities ideally adapted to the respective microorganism as shown in the AFM-image depicted in Fig. 6. The procedure follows industrial standard protocols and can be performed directly on-chip to produce a sensor. When using a QCM device as transducer and exposing it to yeast suspensions with different concentrations, the sensor response shown in Fig. 7 is obtained. Frequency shifts obtained suggest a dynamic sensing range between  $10^4$  to  $10^9$  cells  $\text{ml}^{-1}$ , in the case of shear transverse wave resonators (STW), where the wave propagation is confined within the piezoelectric material thus showing only minor interactions with sur-



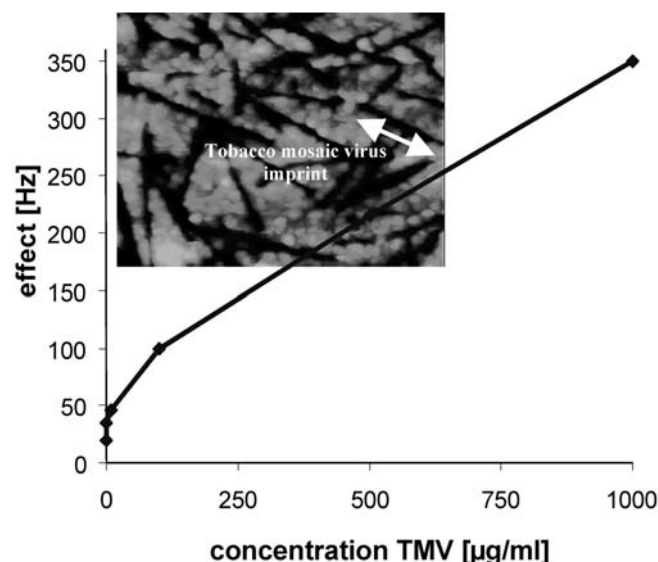
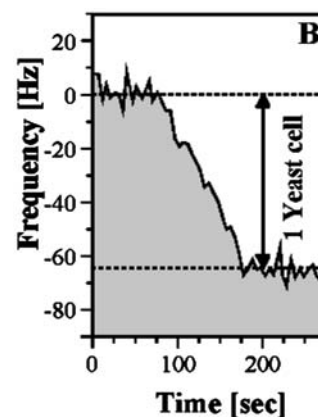
**Fig. 6** Yeast-imprinted polyurethane, some cavities are occupied due to re-inclusion



**Fig. 7** Sensor response of yeast imprint towards different concentrations of analyte

rounding liquid media, even single cells can be detected (see Fig. 8). Studies of the cross-sensitivity of the resulting imprinted sensor layers reveal that the polymers are not only structured in the micrometer range but also show features with nanometer dimensions since even different yeasts could be discriminated by the system. The resulting sensor effects have been found to be highly dependent on the chemical properties of the sensor layer surface: using an excess amount of alcohol substantially increases the sensor effects, which additionally have been found to depend on the pH of the surrounding solution, optimal interaction occurs at  $\text{pH}=6$ . Therefore, obviously the sterical interactions between cell and layer are complemented by the formation of hydrogen bonds thus leading to further improvement of microorganism incorporation into the sensitive material. Finally, the imprinted layers are very robust and therefore can be reused for at least several months. The concept can be extended to the detection of smaller microorganisms such as bacteria and viruses. Especially for the latter, this is a highly promising method as they cannot be seen under a microscope or detected by

**Fig. 8** Frequency response of a 430 MHz shear wave resonator to the adhesion of a single yeast cell



**Fig. 9** TMV-imprinted surface and corresponding QCM sensor characteristic

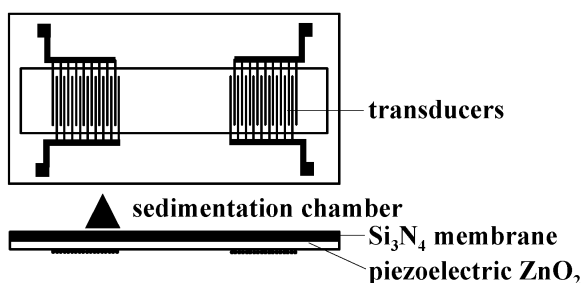


Fig. 10 Setup of the flexural plate wave resonator (Ref. 25)

laser light scattering. In principle, a somewhat similar procedure is used for layer synthesis, as in this case, a surface imprinting procedure is also used. In this case, however, the pits are generated by exposing a prepolymerized material to a suspension of the viruses where they are allowed to sediment. After curing the polymer and removing the viruses from the surface, imprinted cavities as can be seen in Fig. 9 are left behind. The figure also shows the sensor characteristic of such a device reacting towards tobacco mosaic virus.

Some other acoustic devices have been proposed for microorganism detection in liquid phase without an additional sensitive layer. One method introduced by Karube and coworkers [26] consists of two piezoelectric ultrasonic membranes with a sample space between them. One membrane emits ultrasound, the other acts as detector and is connected to an AC-voltmeter. The reading of this has been found to depend on the microorganism concentration of the liquid between the two membranes. By this way  $10^6$  to  $10^9$  cells  $\text{ml}^{-1}$  of *S. cerevisiae*, *B. subtilis* and *Klebsiella* sp. can be detected without the need of an additional sensor layer, of course, no discrimination between different microorganisms is possible. A different ultrasonic method proposed is the so-called flexural plate wave sensor [27] that can be used for the detection of settling *E. coli* cells. It consists of a thin silicon nitride membrane on which transducers are applied that serve as a sender and receiver for an ultrasonic wave, as presented in Fig. 10. Sound propagation velocity in the material is highly influenced by the surrounding media yielding a frequency shift. By this method *E. coli* could be detected with a detection limit below  $8 \times 10^7$  cells  $\text{ml}^{-1}$ .

## Optical Sensors

Optical systems are also very widely spread in chemical sensing, a whole variety of physical parameters (absorbance, fluorescence etc.) is utilized. A small review covering optical methods for cell detection in general (i.e.; not restricted to sensors), can be found in Ref. [28].

A multi-wavelength fluorescence system with excitation wavelengths ranging from 270 to 550 nm and emission spectra recorded between 310 and 590 nm was applied to *Pseudomonas fluorescens* [29]. Simultaneously, headspace gas concentrations were determined on-line, and nitrate, succinate, protein and optical density off-line.

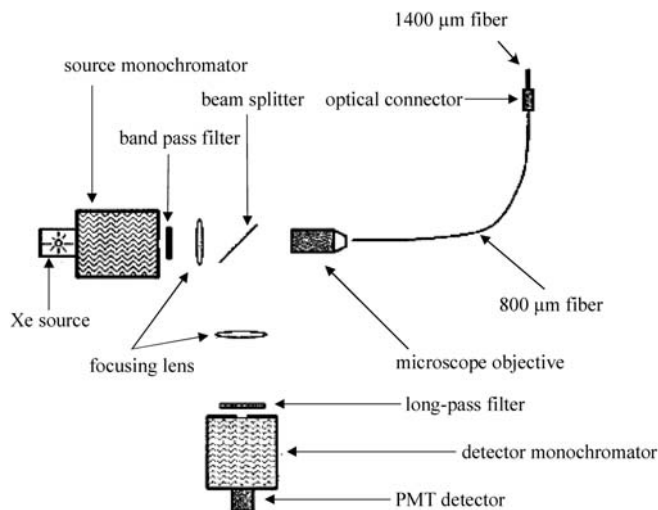


Fig. 11 Fibre optic sensor system for bacteria detection (Ref. 28)

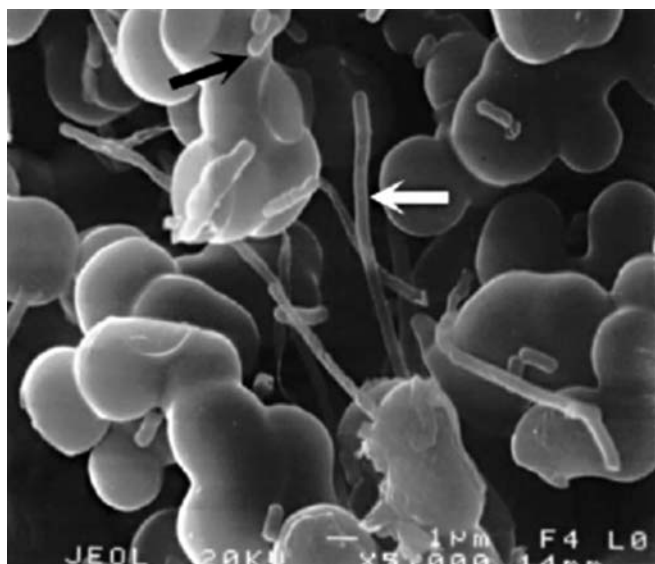
The resulting data set is treated by partial least squares method to predict important cultivation parameters such as the oxygen and the carbon dioxide content in the headspace gas and the content of nitrate and succinate in the breeding solution. None of these compounds actually fluoresces itself, but multivariate data analysis allows to correlate them with the spectra emitted by *P. fluorescens* at different growing conditions.

A fiber-optic sensor for the detection of *Pseudomonas aeruginosa* based on immobilized nucleic acid stains has been proposed by the group of Tabacco [30], the experimental setup can be seen in Fig. 11. An optical fiber with a diameter of 1400  $\mu\text{m}$  is coated with the green fluorescent cell stain SYTO 13 that is a nucleic acid stain binding with DNA- or RNA-strands. On interaction with the analyte species, the dye exhibits an increased fluorescence yield and thus is a highly suitable material for the detection of cells. The sensors prepared can be used to monitor bacteria both in liquids as well as in aerosolized form with reaction times of 15 min and 30 min, respectively, for *P. aeruginosa* a detection limit of  $2.4 \times 10^5$  cells  $\text{ml}^{-1}$  is obtained. This method is much faster than any classical procedure and allows semi-qualitative (different bacterial cells are separated from biological matrices such as serum) and semi-quantitative determination (precision less than a magnitude, dynamic range about 3 decimal powers). The same group also reported the use of a fluorescence system in combination with a hydroxy-terminated polyamidoamine-dendrimer [31]. SYTOX green fluorescent nucleic acid stain is regarded as a stain for dead cells, as it usually cannot permeate plasma membranes of intact microorganisms. Combining it with the aforementioned dendrimer in a sensor layer, *P. aeruginosa* can be detected down to  $5.7 \times 10^5$  cells  $\text{ml}^{-1}$ , fluorescence intensity is enhanced by 350% compared with sensors coated only with the dye while omitting the polymer. A further advantage of using the dendrimer is its stabilizing ability on the sensor system as it prevents decomposition of the biogenous sensing materials: the resulting instruments retain their sensing

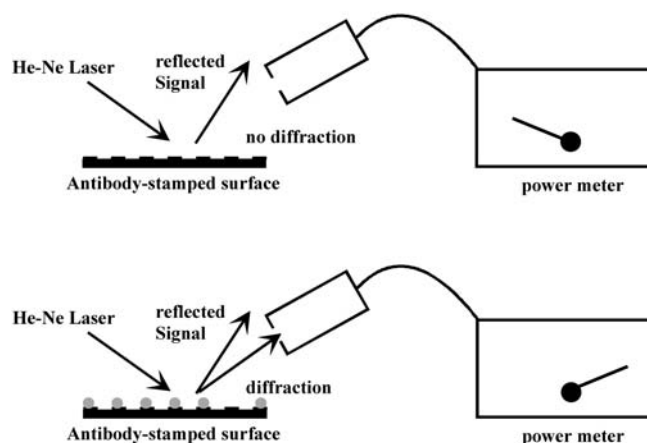
abilities even after drying and desiccation thus leading to a fast assay for bacterial contamination.

Fiber optical sensors have also been proposed for the detection of viruses, e.g., for the Newcastle disease virus [32] and for human measles virus [33]. Both works include the coating of a fiber optic sensor with antibodies against the virus, in both cases a polyclonal antibody from a rabbit is applied, the device itself is both a suitable transducer as well as a concentration chamber. For the Newcastle disease virus, fluorescein-labeled antibody is bound to the reacted viruses and the resulting fluorescence intensity is measured. A gene technological approach for detecting the presence of viruses is given by the group of Norris [34]. They use genetically modified *E. coli* for the detection of bacteriophages in aerosolized form, the bacteria express green fluorescent protein (GFP) when infected. To achieve this, the bacteria are immobilized in a macroporous aerogel fabricated by acidic condensation of tetraethoxysilane followed by drying in supercritical  $\text{CO}_2$ , finally, disks with a thickness of 1 mm are prepared from the resulting materials. These are first soaked with phosphate-buffered saline followed by flowing a bacterial solution through them and thus immobilizing the microorganisms. When exposed to aerosols of bacteriophages, the fluorescence increases. A detection limit of  $10^5$  infectious units per ml in the original solution is achieved within four hours, Fig. 12 shows a SEM image of the colonized aerogel surface including both healthy and infected bacteria.

A completely different transducer approach is given by a group at Cornell University [35]: here microcontact printing is used to generate an antibody grating on a surface. When this is exposed to bacteria, light scattering can be observed, which is not present in the absence of microorganisms (see Fig. 13). Monitoring the light intensity of the first-order scattered light allows for the detection of



**Fig. 12** Aerogel containing healthy and infected *E. coli* bacteria (Ref. 32)



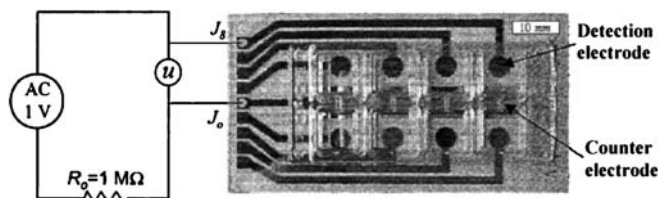
**Fig. 13** Schematic diagramming of a light scattering sensor (Ref. 33)

*E. coli* in a concentration range between 250 to 450 cells  $\text{cm}^{-2}$ . This group printed the antibody directly onto an untreated silicon surface, however, the transducer interface is often derivatized. An extensive study of surface modifications and different stamping procedures as well as several printed patterns is given in Ref. [36]. After trying a wide variety of immobilization protocols the direct microcontact printing of suitable derivatized antibodies gave the best results. This method ensures stable, homogeneous covering of the transducer surface hence is ideal for binding microorganisms. Testing of the resulting sensors with *E. coli* as a model compound for method sensitivity yields a detection range of  $10^5$  to  $10^9$  cells  $\text{ml}^{-1}$ .

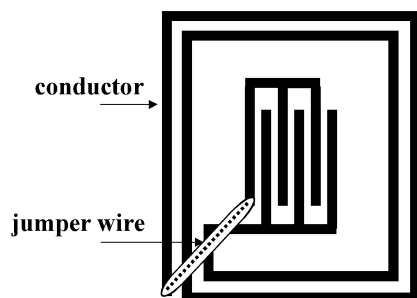
## Impedance Sensors

Capacitive measurements of microbial growth are already used in industry to assess contaminations, although data evaluation can be somehow empiric. An in-depth study on different factors influencing this measurand, such as temperature fluctuations, ionic strength and pH of the medium etc. is given in Ref. [37]. For example, the conductance and susceptibility of the medium is effected by temperature as a result of the changing liquid viscosity. Although an increasing microorganism concentration should yield a proportional change of the observable admittance as a result of decreasing contact between electrodes and ions, this effect cannot be observed for cell densities between  $10^1$  and  $10^7 \times \text{cells ml}^{-1}$ . The authors therefore suggest using capacitance rather than conductance for cell sensing.

Microorganisms have a great influence on the impedance of defined measuring chambers because the lipid bilayer forming the outer membrane acts as an insulator. Luong et al. [38] have proposed a sensor system making use of this phenomenon: they constructed an array of eight small gold electrodes deposited on the bottom of tissue culture wells with a suitable counter electrode and immersed the whole system in a culture medium (Fig. 14). When cells attach to the electrode surface, the impedance



**Fig. 14** Eight-electrode array for impedance measurements with cells (Ref. 38)



**Fig. 15** Print plate design of a remote-controllable LC-sensor (Ref. 39)

increases and reaches a saturation value as soon as a monolayer is formed, however, the signal continues to fluctuate as a result of cell desorption and re-adsorption. This fluctuation ceases instantaneously when cytotoxic compounds such as  $\text{Hg}_2\text{Cl}_2$  are added to the system. Cells are rapidly killed and thus remain adsorbed on the surface without further motion, which gives the method high potential for the assessment of compound toxicities [39]. All measurements are carried out on-line, the results are obtained in real-time. The group also published an in-depth study of the theoretical framework behind this measuring technique [40]. A remote-operable sensor for cell growth was proposed by the Grimes group [41]: the authors suggest an inductor-capacitor resonant circuit on a print plate (Fig. 15) and placing it in the culture medium, sensor interrogation is done via an external antenna loop, aligning between the sensor and the antenna is not necessary. When microorganisms grow on the surface of the device, the capacitance of the interdigital transducer is changed leading to an overall change in permittivity and thus a detectable shift in resonance frequency of the LC-oscillator. The authors give a detailed description of the theoretical background of the system as well as sensor curves for *B. subtilis*, *E. coli*, *P. putida* and *S. cerevisiae*. During the growth phase, the culture permittivities are continuously monitored, as a resolution of  $\Delta\epsilon=0.01$  can be achieved, a time interval of five seconds can be used during operation. During a regular breeding procedure, sensor responses increase with culture time monotonously, any deviations in this behavior indicate the presence of cytotoxic compounds. Usually, impedance measurements yield a sum signal including both the influence of the matrix and of the microorganisms. To address the suspended particles exclusively in solution, AC electrokinetic methods can be applied which are based

on the difference in polarizability between particle and medium and thus make use of differential phenomena [42, 43]. Variations in electrode geometry allow different manipulations of the cells, if homogeneous fields are applied deformation, orientation and attraction can be observed, in the case of inhomogeneous fields, dielectrophoresis, trapping, electrorotation and traveling wave dielectrophoresis. Naturally, the phenomena cited can also be used for biochemical sensing applications.

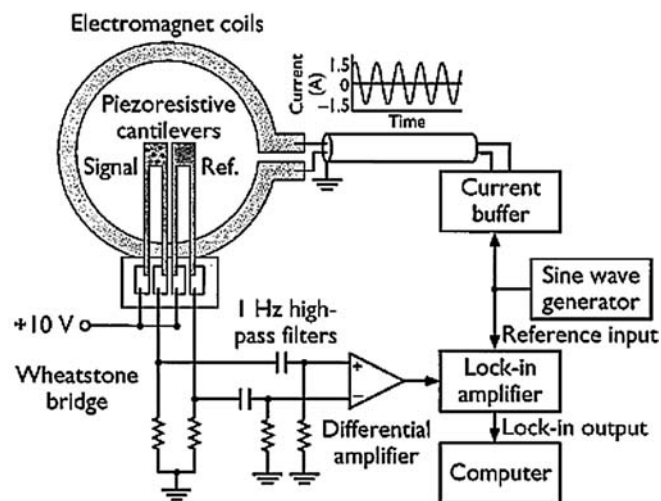
Wolf and co-workers [44] propose a sensor array consisting of ion-selective field effect transistors and several auxiliary measuring probes, such as sensors for temperature, oxygen and cell metabolites, as well as the possibility of accessing the sample with light microscopy. They mainly detect extracellular pH but also other metabolic by-products of bacteria to estimate the activity and type of microorganism present. The system, for example, has proven to be an outstanding tool for the rapid on-line determination of physiological aspects of different drugs on the respective cell population. In another study, ten different MOSFET sensors were combined into an array [45] for the determination of bacterial infections in mammalian cell cultures by monitoring of the headspace gas above the fermentor. Infections result in a significant change in the signal pattern in the array about ten hours before the classical method of monitoring the  $\text{pO}_2$  yields a detectable frequency shift. Thus the system is highly suitable for on-line monitoring in industrial process control as it allows for fast and early countermeasures in the case of contamination.

Light-addressable potentiometric sensors are another type of transducer that have high potential in biological sensing. These devices consist of an electrolyte-insulator-semiconductor (usually  $\text{Si}_3\text{N}_4/\text{SiO}_2/\text{Si}$ ) structure, where the thickness of the depletion layer greatly depends on the local value of the surface potential, which, of course, is influenced by the surrounding environment. The resulting capacity is read out by the AC-photocurrent of an intensity-modulated light-diode. Detection limits of the method are very low, for *Yersinia pestis* one single cell is observed as well as some ten spores of *Bacillus subtilis globigii* [46]. Modification of the  $\text{Si}_3\text{N}_4$ -surface leads to highly improved recognition abilities and specificity of the system [47].

## Other Sensors

A further transducer system has been introduced that can be regarded as a spin-off of the fast-evolving AFM-technique for nanometer-resolved surface characterization [48]. In this case, a cantilever brought to resonance with a piezo drive is put into the coil of an electromagnet. The tip is modified by an immobilized antibody interacting specifically with analyte cells (for the setup see also Fig. 16). After incubating and washing off the non-reacted microorganisms, magnetite crystals bound on antibodies are reacted with the cells. Turning on the electromagnet thus yields a change in cantilever deflection that is detected electronically. The





**Fig. 16** Sensor design for microorganisms based on AFM cantilevers (Ref. 46)

setup reaches a detection limit of about  $10^{-18} \text{ mol l}^{-1}$ , which is about eight magnitudes better than ELISA.

## Conclusion

In this review we have presented the progress made in the field of microorganism sensing during the last decade. The most straightforward strategies to fulfill the analytical needs are given by using bare transducers, either optical, mass-sensitive or electrochemical ones, as the presence of cells usually changes several physical parameters in the vicinity of the sensitive area. Most of these sensors rely on biofilm formation on the sensitive area and thus usually show satisfying sensitivity. Nonetheless, they are not suitable for discriminating between different types of microorganisms thus they lack the selectivity necessary for a powerful chemical sensor system. This can be introduced by utilizing methods and chemical reactions that are already applied for the determination of biogenous species, namely immunochemical interactions. Selective or even specific antibodies can be deposited on the sensitive areas of the respective transducers leading indeed to powerful sensor systems. Different protocols with variations of the layer chemistry are used, most surface modifications aim at increasing the stability of the chemical bond between the antibody and the transducer. Although very powerful, these systems have the drawbacks of limited life-time and re-usability, because antibodies as representatives of biogenous compounds and are usually prone to denaturation products and often show irreversible binding of the microorganism of interest. The resulting limitations can be addressed by replacing the biological coating material by synthetic biomimetic polymers that should act as artificial antibodies. Surface imprinting procedures, where the microorganisms are cast with organic or inorganic polymers is mainly used for this purpose. After finishing the layer synthesis steps, the template cells are washed away leav-

ing behind adapted cavities for their re-inclusion. These sensor-layers show sensitivities similar to natural antibodies thus mimicking nature excellently. As they are a product of modern polymer chemistry, they are much less sensitive to degradation and harsh environments than their natural counterparts. Combining highly specific biological interactions with chemical skills thus leads to very powerful analyzing systems for bioanalytes in general and especially microorganisms of many different shapes, types, and sizes.

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