# Chapter 2
# Objectives

Biosensors based on whole cells as the biological recognition unit (CBBs) have gained an increasing interest in the past years by research institutes focusing on the development of new technologies that can serve as basis for the label-free and non-invasive screening of living cells. Due to steady technical improvements in terms of throughput and handling, progresses in data analysis and interpretation, and the development of numerous cell-based assays, there is also an increasing interest in CBBs on the part of the pharmaceutical, food and biosecurity industry [1–6]. In CBBs, cell lines can serve as a renewable biological recognition element for biomedical assays, such as pathogen and toxin detection and drug discovery [7]. Cells cultured *in vitro* can mimic on a small scale the functional effect of any substance or stimulus on tissue, organs or the whole organism and human body, respectively. Thus, biosensing of cells provides meaningful high-content information in a physiological context, which is not accessible by interaction studies of isolated biomolecules or by label-based and therefore invasive cell-based assays. In terms of drug discovery, for instance, the application of CBBs allows a pre-selection of candidate test substances and concentrations in preclinical screening studies to minimize the number of animal tests, which is desirable for both ethical and cost reasons.

Figure 2.1 gives an overview of the technical state of the art of the three main CBBs used for monitoring structural changes of cells, QCM, ECIS, and SPR, with respect to available modes of operation and sensor combinations (indicated with a connecting line) at the beginning of this thesis. Sensing modes are indicated with blue arrows directed away from the sensor principle and actuating modes are highlighted in red with arrows pointing inwards to the cells. The stimulation of cells and their microscopic investigation, mostly used in addition to CBB, are also illustrated for the sake of completeness. Figure 2.2 shows the same overview, however, expanded with the contributions of this thesis (highlighted with blue borders). Methods and applications not used or not presented in this thesis are shaded in grey.



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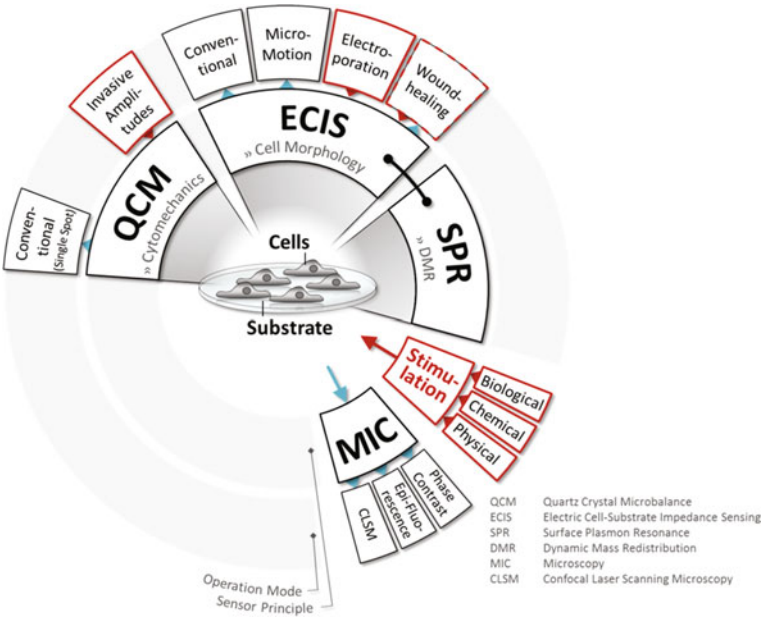


**Fig. 2.1** State of the art of (combined) CBBs for monitoring structural changes of cells at the beginning of this thesis

It was the aim of this thesis to improve and develop real-time sensor devices that can be used to monitor assays with living cells label-free and non-invasively on the basis of the quartz crystal microbalance (QCM) technology. One focus thereby was set on the development of a multi-spot QCM sensor (**MQCM**), which should be designed, characterized, and optimized. The MQCM should be finally applied for investigating the mechanical properties of mammalian cells and their changes by analysis of the QCM impedance of cell-covered quartz resonators. Thus, the QCM should be established as a method that enables the continuous monitoring of cytomechanics under physiological conditions, with a high time resolution, manageable technical expenses, and a moderate throughput in cell-based screenings. This should be evaluated in various studies with compounds that affect the micromechanics of cells.

Another project aimed on the integration of the ECIS principle into the surface of the MQCM disks developed, in order to provide a novel hyphenated (**QCM-ECIS**) sensor combining piezoelectric and electrochemical transduction in one experimental setup. This should enable the label-free readout of cell morphology changes as well as cell-cell and cell-substrate interactions by means of ECIS in parallel to the viscoelastic profiling of cells by QCM recordings. The dual sensor platform should be developed using thin film technology and standard photolithography. An optimal electrode layout of the QCM-ECIS sensors should be evaluated, by means

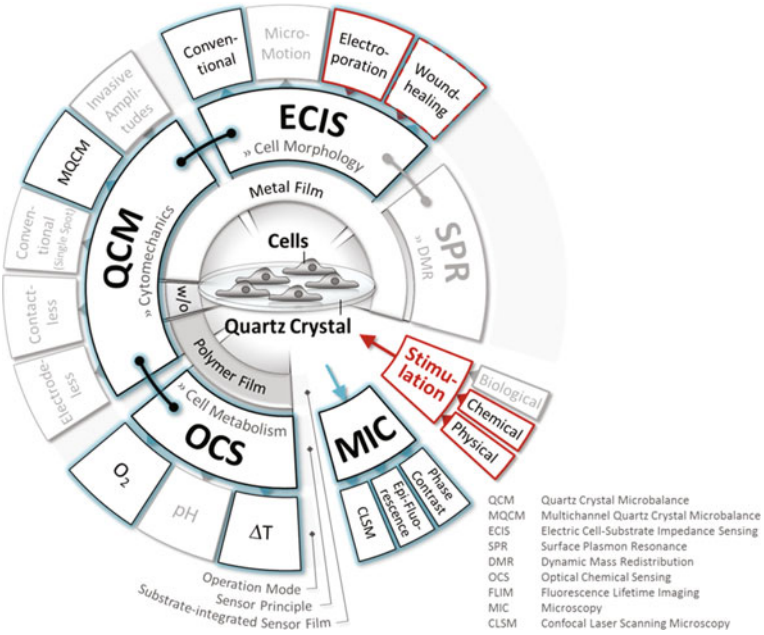


**Fig. 2.2** State of the art of (combined) CBBs for monitoring structural and metabolic changes of cells at the end of this thesis

of microscopic investigations and analysis of the impedance characteristics of both QCM- and ECIS-mode readouts. Once a system suitable for cell-based biosensing applications is identified, the sensing performances of the individual transducer principles of the dual sensor should be evaluated on the basis of various cell adhesion and cell stimulation assays and, as a key issue, the added value of the hyphenated approach should be identified and discussed with respect to the separate sensor setups and readings.

In a third project the QCM should be combined with luminescence-based OCS in order to provide another class of sensor combinations (**QCM-OCS**) for the purposes of multi-parametric CBB. A first QCM-OCS combination aimed on the experimental determination of temperature increases on the QCM surface, which are expected if the resonator is operated at elevated driving voltages and oscillation amplitudes, respectively. Two-dimensional OCS thereby should be implemented by means of a temperature-sensitive paint (TSP) and fluorescence lifetime imaging (FLIM). In another approach, an oxygen-sensitive sensor film should be developed which at the same time should serve as the growth substrate for mammalian cells. The cytocompatibility of the pressure-sensitive paint (PSP) used for this purpose should be investigated by means of QCM measurements and a PSP-coated substrate compatible for cell layer establishment should be realized. Finally, proof-of-concept

studies should demonstrate the capability of the chemosensitive transducer for label-free and time-resolved sensing and imaging the oxygen consumption rate (OCR) of a cell layer upon stimulation. This should provide the basis for the substrate-embedded screening of further analytes, such as for instance $H^+$ or glucose, in cell-based assays by means of OCS.

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