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Development of an automated system for the analysis of cell-free fetal DNA from maternal plasma for non-invasive pre-natal diagnostics

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The analysis of circulating cell-free (cf) DNA from plasma, serum or urine has the potential to serve as non-invasive approach to detect and monitor targets associated with certain diseases. In 1997, the presence of fetal DNA in the plasma and serum of pregnant women was demonstrated. This opened new perspectives in field of non-invasive pre-natal diagnostics since the analysis of cell-free fetal (cff) DNA can provide information about pregnancy related disorders (pre-eclampsia, preterm labor), chromosomal aberrations (e.g. aneuploidies), and genetic disorders (e.g. cystic fibrosis, thalassaemia, Huntington's disease). We report on the development of an automated and integrated modular system for the isolation, amplification and detection of cffDNA from maternal plasma for non-invasive pre-natal diagnostics. The system consists of a first module for the cfDNA isolation from plasma based on silica-coated magnetic beads technology. Subsequently, the cfDNA obtained is introduced to a second module which is based on a polymeric microsystem containing a capillary electrophoresis step for the size separation of the fetal DNA from maternal DNA. Finally, the cffDNA is transferred to the amplification/detection module. This module consists of PCB (Printed Circuit Board) electrode arrays functionalized with surface immobilized primers for the multiplexed isothermal recombinase polymerase DNA amplification and electrochemical quantitative detection of specific genetic sequences. The developed technology is of generic and flexible nature allowing its facile modification to other targets of interest in clinical diagnostics and thus the developed platforms can also be exploited for analysis of circulating nucleic acids in oncology and multiple other disorders.

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Optical fibers and light in biomedical application

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Two optical fiber applications for in vivo studies, namely, recording of ciliary beating frequency in respiratory epithelia and analysis of fluorescence spectra from living cells have been studied. First, a method based on a "pigtail" semiconductor laser emitting at 642 nm and using SM and MM fibers has been developed. Backward scattered signals recorded from the pig trachea were evaluated by fast Fourier transform. The detected beating frequency and its temperature dependency are in a good agreement with data obtained with other non-fiber optic devices. This opens the possibility to adapt the fibers for endoscopic studies. Second, absorption and fluorescence spectra were recorded from a "simplified cell" - a mixture of dominant cell fluorophore: (NADH, pyridoxine and tryptophan) at different concentrations and pH. Calibration curves for NADH concentration were derived and emission spectra, deconvoluted by SW GASPED method, were compared with those of individual pure fluorophore. Spectrometer SHIMADZU UV-3600 (absorption spectra) and a Fluoromax-4 fluorimeter were used. The same approach was then adopted for cultures of living baker yeasts by using excitation at 340-365 nm. In agreement with literature, the NADH fluorescence (at 460 nm) responded to metabolic conditions imposed. These results indicate that the use of optical fibers may be a valuable tool for studies of cell viability *in vivo*, for example, in the domain of photodynamic therapy.

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