A Sensor Concept for Label-Free Cell Analysis

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Based on IR absorbance spectra of healthy and malignant breast [1] and blood cells [2] found in literature we investigated the possibility of disease stage cell discrimination by comparing only a few absorbance peaks. This way no expensive FTIR spectroscope or time consuming expensive staining/labeling steps are required. Absorbance differences between healthy and malignant cells at wavelengths 3.42 and 3.51 µm (corresponding to lipid CH_2-anti-symmetric and CH_2-symmetric stretch respectively), showed a possible disease stage discrimination basis. To test the few wavelengths hypothesis the absorbance spectra of healthy and carcinoma epithelial kidney cell lines were recorded with an FTIR-spectroscope and compared. The results showed a significant difference between the three cell lines which have led to the development and realization of a novel sensor system.

Introduction

Distinguishing healthy from malignant cell types by means of labeling and staining is time consuming and expensive. Also highly trained personnel are necessary to interpret the obtained data. Infrared spectroscopy, IR absorbance due to specific molecular vibrations, is an interesting diagnostic tool without the need of added labels. Instead of recording the whole IR spectra between 2 and 20 µm with a Fourier transform infrared (FTIR) spectroscope a few wavelengths in the lipid absorption region (between 3 and 4 µm) could be sufficient to distinguish healthy from tumor cells. This way, a smaller and cheaper sensor system based on LED light sources, narrow bandpass filters and a room temperature operable photodiode detector could be used for label-free cell analysis. By comparing the IR absorbance spectra of healthy and malignant cells, published in literature, we hypothesized a few wavelength based cell type discrimination concept. The concept is based on the absorbance ratio between lipid CH_2 antisymmetric and symmetric stretch (respectively at 3.42 and 3.51 µm). To test the few wavelength hypothesis and get better understanding how the IR absorbance spectra are recorded and compared we made our own data set of healthy (MDCK) and carcinoma (A-498 and Caki-1) epithelial kidney cell lines.

Experimental

Few Wavelength Concept

The investigated cell lines were all grown under the same conditions in monolayer on IR transparent calcium-fluoride (CaF_2) slides. Just before the absorbance recordings the cells were vacuum dried. In Fig. 1 (a) the averaged absorbance spectra of four

MDCK, A-498 and five Caki-1 sample spots are shown. Due to artifacts such as differences in water concentration and sample thickness, normalization and baseline correction are needed when sample comparison is required. Figure 1 (b) depicts how the ratio (CH_2 symmetric / antisymmetric stretch) can be extracted for sample comparison with baseline correction.

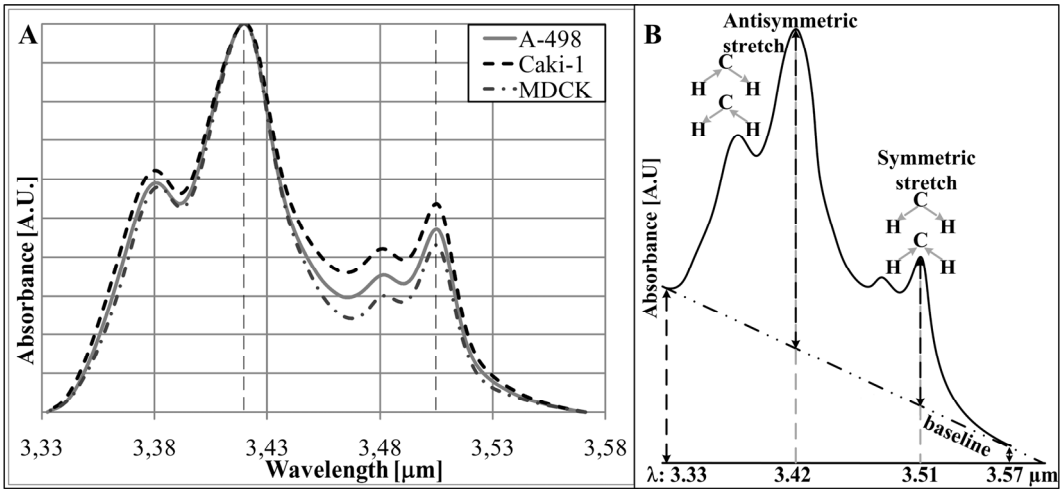

Fig. 1: (a) Normalized and baseline corrected IR Absorbance spectra comparison of epithelial kidney cells MDCK, A-498 and Caki-1 between wavelengths 3.33 and 3.57 µm recorded with a Bruker Equinox 55 spectrometer (240 scans per spectrum, 4 cm^{-1} resolution and 1 mm beam diameter). The absorbance peak at 3.51 µm is increased in the two carcinoma cell lines (10% A-498 and 25% Caki-1) compared to the healthy MDCK cell line. (b) Ratio determination. From each sample the absorbance at 3.33 and 3.57 µm is used for the base line (dashed black arrows) calculating the absorbance ratio 3.51/3.42 µm (dashed black grey arrows).

The measured differences in absorbance ratio (10% and 25% increase compared to MDCK for A-498 and Caki-1 respectively) confirms the hypothesis that cell types can be distinguished by means of the few wavelength concept. The next step is to design a sensor system.

Sensor Concept

To cover the four wavelengths two LEDs are used in combination with two narrow bandpass filters (NBP) each. The transmittance spectra are shown in Fig. 2. Figure 3 (a) shows a sensor concept consisting of two LED IR sources, four NBPs, a beam-splitter to focus both beams at the same spot, and a detector.

Compared to liquid nitrogen cooled detectors used in FTIR spectroscopes, for the 3.3 to 3.6 µm wavelength range there is a room temperature operable photodiode (PD) based detector available. A sensor setup in early development stage is shown in Fig. 3 (b). With this setup absorbance ratio measurements of yeast samples have been made.

First Sensor Measurement

Dried baker's yeast was resuspended in phosphate buffered saline (PBS). A 2 µl sample was pipetted on a CaF_2 slide. Before measuring the absorbance ratio the sample was dried for 2 hours above a heated plate at 50 °C. The IR beams of both LEDs were

focused on a 1.5 mm diameter aperture. The CaF_2 slide was positioned right after this aperture. To determine the absorbance ratio first the 100% transmittance voltages (PD output current converted to a voltage, amplifier) were measured by using an empty CaF_2 slide. The next step was to measure and calculate the % absorbed values of the yeast sample at the four wavelengths (Fig. 4) and subtract the baseline at 3.42 and 3.51 µm.

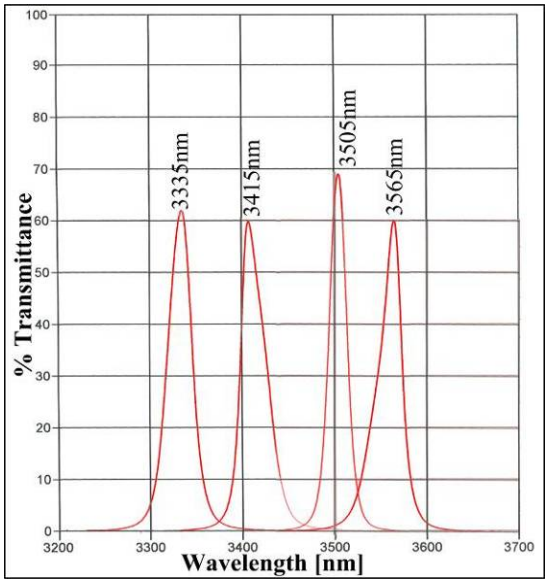

Fig. 2: Transmittance spectra of the four used NBP filters.

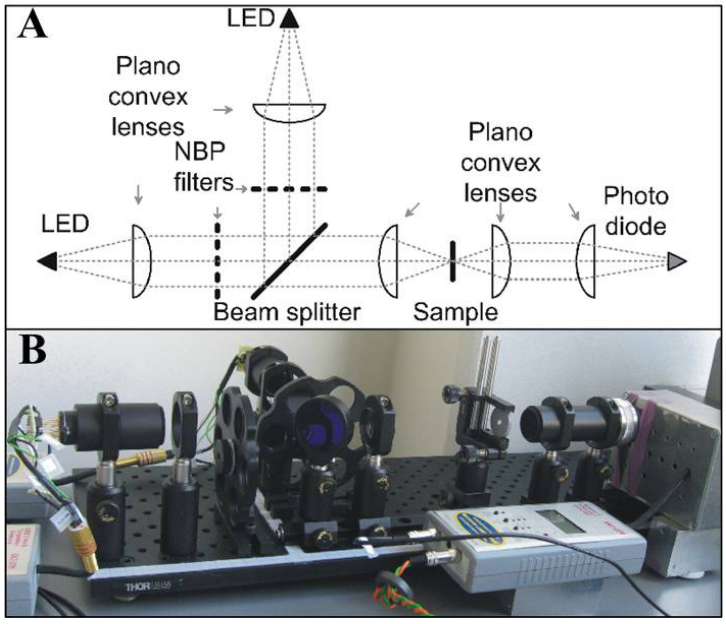

Fig. 3: (a) Sensor concept. Two LEDs operated in alternating pulse mode both emitting in a broad range so that with changeable narrow band pass filters (NBP) two wavelengths can be used (3.33 and 3.42 µm for LED1 and 3.51 and 3.57 µm for LED2). A beam-splitter is used to focus the two beams on the same spot of the sample. The photodiode (PD) is used as detector and can be operated at room temperature. (b) First realized sensor setup.

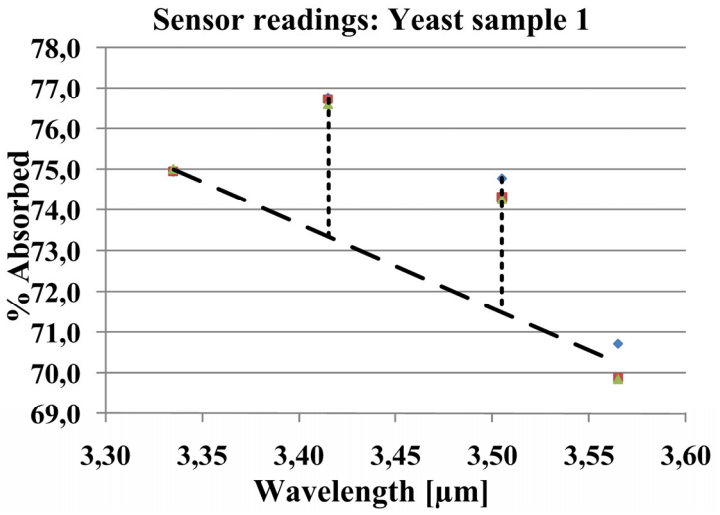

Fig. 4: IR absorbance sensor data of a yeast sample measured three times. The average measured absorbance ratio (3.51/3.42 µm) is 0.89.

The absorbance ratio of a 2 hours dried yeast sample, calculated as previously described, was 0.89. One wavelength absorbance value consists of the averaged value of 1000 measurements. Of each 8 µs LED pulse, 12 samples were recorded; due to background noise, rise and fall time the four second highest values were averaged to get one measurement value (one "% absorbed/wavelength" point shown in Fig. 4 is an averaged measurement value of 1000 times 4 sample values). The same sample measured again 24 hours later showed a lower absorbance ratio 0.64.

Discussion

The difference between the measured yeast IR absorbance ratios after 2h and 24h drying is most likely due to a higher fraction of evaporated water. A ratio of 0.65 was measured earlier of a vacuum dried yeast sample with an FTIR spectroscope. When the absorbance ratios of different samples have to be compared, constant sample preparation conditions are of importance.

Conclusions

The IR absorbance ratio (3.51/3.42 µm) measurements with a FTIR spectroscope of healthy MDCK and carcinoma A-498 and Caki-1 epithelial kidney cell lines, confirm that it is possible to discriminate between cell types by using the few wavelength concept. First sensor measurements showed detectable peaks of interest.

Outlook

The next step to test the sensor setup is to measure and compare the IR absorbance ratio of MDCK, A-498 and Caki-1.

Acknowledgements

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