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# *In situ* Fourier transform infrared analysis of live cells' response to doxorubicin

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ABSTRACT

The study of the response of cancer cells to chemotherapy drugs is of high importance due to the specificity of some drugs to certain types of cancer and the resistance of some specific cancer types to chemotherapy drugs. Our aim was to develop and apply the label-free and non-destructive Fourier transform infrared (FTIR) method to determine the sensitivity of three different cancer cell-lines to a common anti-cancer drug doxorubicin at different concentrations and to demonstrate that information about the mechanism of resistance to the chemotherapy drug can be extracted from spectral data. HeLa, PC3, and Caco-2 cells were seeded and grown on an attenuated total reflection (ATR) crystal, doxorubicin was applied at the clinically significant concentration of 0.1–20 μM, and spectra of the cells were collected hourly over 20 h. Analysis of the amide bands was correlated with cell viability, which had been cross validated with MTT assays, allowing to determine that the three cell lines had significantly different resistance to doxorubicin. The difference spectra and principal component analysis (PCA) highlighted the subtle chemical changes in the living cells under treatment. Spectral regions assigned to nucleic acids (mainly 1085 $cm^{-1}$) and carbohydrates (mainly 1024 $cm^{-1}$) showed changes that could be related to the mode of action of the drug and the mechanism of resistance of the cell lines to doxorubicin. This is a cost-effective method that does not require bioassay reagents but allows label-free, non-destructive and *in situ* analysis of chemical changes in live cells, using standard FTIR equipment adapted to ATR measurements.

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## 1. Introduction

Chemotherapy is one of the main treatments of cancer. However, as cancer cells can be sensitive or resistant to chemotherapy agents, drug resistance is one of the major obstacles for a successful treatment. It is important to develop methodologies to evaluate the interaction of cancer cells with chemotherapy drugs so that the mechanism of drug action and drug resistance can be better understood for the development of more effective drugs. Knowing the response of cancer cells to chemotherapy drugs may also allow medical practitioners to make an informed choice for the most suitable drugs for each individual patient. Ideally, the analysis method would allow a fast and effective prediction of the sensitivity of cancer cells to drugs, as well as provide information on the mechanism of action of the drug or the resistance to it at a low running cost.

Fourier transform infrared (FTIR) spectroscopy is a non-destructive and label-free technique that provides data related with the chemical composition of the samples under analysis. It is increasingly becoming an important analytical tool for biomedical research [1]. The FTIR spectra of human cells are typically dominated by the absorbance due to their content in proteins, lipids, carbohydrates, nucleic acids and phosphorylated compounds. Subtle differences in cell spectra have been used to distinguish cells in different stages in the cell cycle [2], apoptotic cells [3] or cell lines [4,5], to identify cancer cells [6], and to predict the aggressiveness of cancer cells [7]. Several studies have shown a relationship between the mode of action of chemotherapy drugs and changes in FTIR spectra of dried cells [8–10], as well as differences in FTIR spectra of drug-resistant and drug-sensitive cells [11–13]. In these studies cells were treated with a mildly cytotoxic or cytostatic concentration of drug, generally the $IC_{50}$ value for the drug toxicity, and were then collected and dried prior FTIR analysis, as water causes a strong interference in FTIR analysis. The drying process however may cause alterations or artefacts. Also, cells are generally harvested with trypsin, including in some live cell studies [14]. Trypsinising cells has been shown to change the protein composition in the plasma membrane, as well as regulating apoptosis related gene expression [15]. After trypsinisation the cells may recover after a few hours in medium [15], but in most studies the cells are resuspended in isosmotic saline solution and are not allowed to settle before analysis even though significant differences in cell spectra have been previously reported. The trypsinisation and drying processes may mask or eliminate biochemical differences caused by the treatment with chemotherapeutical agents.

Another approach is to measure living cells with FTIR while they are still attached to a substrate to mimic the environments in most *in vitro* cell studies. This can be achieved by using the attenuated total reflection

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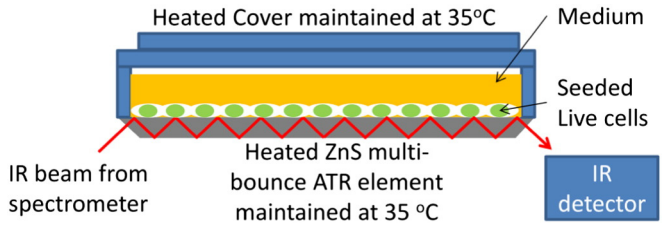


**Fig. 1.** ATR element and cell culture setup for live cells' FTIR measurement.

(ATR) measurement method where live cells are seeded with culture medium and allowed to reach confluence on the measuring surface of the ATR element. A number of previous work had demonstrated that this can be a powerful method to study living cells including study of cell death [16] cell activation [17,18], differentiation [19], cell adhesion [20,21], growth inhibition [22], and drug accumulation [23]. Using this method, only the attached live cells are measured with no significant contribution from the medium above the cell because the depth of penetration of the measurement is relatively small, typically of 2–3 μm from the surface of the ATR element. This is smaller than the thickness of a living cell. Note that the region of the cell that is closer to the ATR element will contribute more to the absorbance than the region of the cell that is further away from the ATR element such that there is an opportunity to profile the chemical difference along the thickness of the cell [24,25].

Here we propose to apply the ATR FTIR method, for the first time, to measure the effects of chemotherapy drug doxorubicin in three different human cell lines at different drug concentrations, *in situ*, using settled live cells in culture to focus on the biochemical alterations caused by the drug. The three different cell lines were chosen with different degrees of resistance to the drug to demonstrate that ATR FTIR can be a fast and cost effective method to determine the sensitivity of cancer cells to drug and at the same time providing information on the mechanism of drug resistance. We have validated the drug sensitivity results using standard MTT assays as well as complementary fluorescence measurements.

## 2. Materials and methods

### 2.1. Multi-bounce ATR FTIR accessory

A temperature controlled multi-(*i.e.* 10) bounce ATR accessory trough plate (HATR, Pike technologies) and a 45° ZnS ATR element (80 mm × 10 mm × 4 mm, Crystran Ltd., UK) were used. The path length in the living cells produced from this accessory is approximately 20–

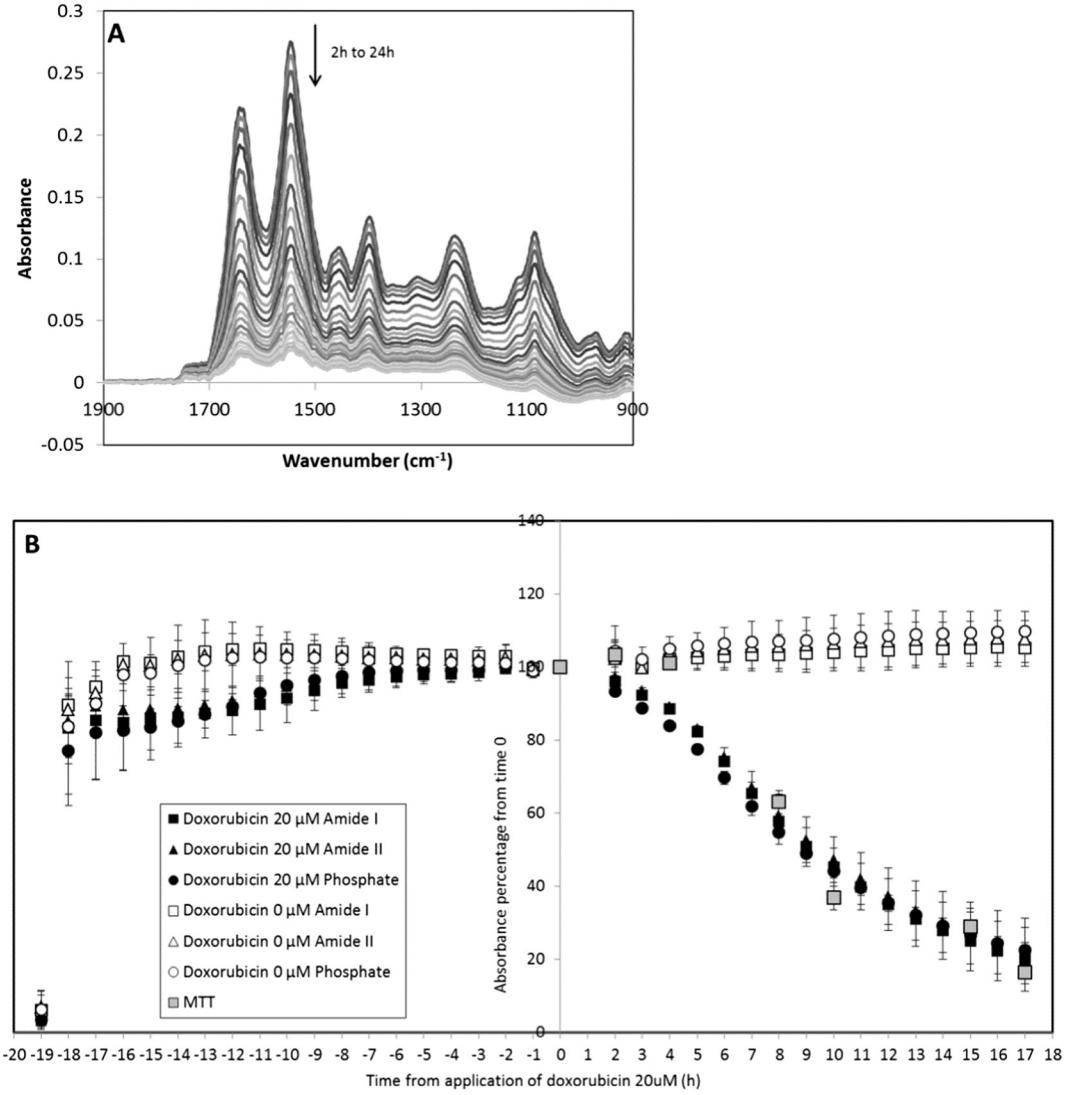


**Fig. 2.** Effect of the addition of 20 μM doxorubicin to FTIR spectra of HeLa cells. (A) FTIR spectra of HeLa cells in the 24 h following the addition of doxorubicin, (B) intensities of the amide I (1645 $cm^{-1}$), amide II (1550 $cm^{-1}$) and phosphate mainly from the DNA backbone (1085 $cm^{-1}$) from the moment the cells were seeded, normalised at the intensity value when doxorubicin was added (intensity at time 0 = 100%), plotted together with the cell viability by the MTT assay.

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