Experiment Proposal

Experiment Number: 4333

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Experiment Title	Detecting drug effects by Fourier Transform InfraRed microspectroscopy (microFT-IR) on single cells.		
Beamline	**B22**	**Shifts Requested:** 15	
Industrial Collaborators	No		
Access Route	Direct Access - New	**Previous RB Number:** -	
Science Areas	Medicine, Biology and Bio-materials, Technique Development		
Sponsored Grant	No	**Sponsor:** -	
Grant Title	-		
Grant Number	-		
	Start Date: -	**Finish Date:** -	
EU Access?	Italy	**Similar Submission?**No	

Abstract

We will exploit some potential advantages of Fourier Transform InfraRed absorbance microspectroscoy (microFT-IR) to identify biochemical and morphological effects of drugs by SR IR microanalysis on single cells. The goal is to start developing a reference library of validated FT-IR spectra via Synchrotron Radiation IR microprobe for the future application of microFT-IR analysis as a drug screening test and for clinical follow-up of patients with leukaemia. We expect to acquire the maximal number of representative spectra with acceptable signal-to-noise ratio (S/N) values for statistics significance in samples. In parallel, complementary biochemical information achieved in samples will be associated with spectral information allowing to cross-validate reference FT-IR absorbance spectra of functional and structural cellular events induced by drugs in cancer cell models.

Publications

Diamond Sample record sheet

Principal contact	Mr G Bellisola, giuseppe.bellisola@univr.it, Tel: +39 045 8126451
Instrument	B22, 15 shifts, preferred contact is (none specified)
Special requirements	The usage of peripheral lab for the completion of sample preparation.

SAMPLES

Samples are now detailed on a separate excel file which can be obtained from the online proposal system.

Science Case: Detecting drug effects by Fourier Transform InfraRed microspectroscopy (microFT-IR) on single cells. **Proposer**: Dr. Giuseppe Bellisola, The University of Verona, The Department of Pathology, Italy.

Aims of the experiment and specific scientific background. There is great interest to develop new tests that may assist in determining the prognosis and optimising the therapy of human chronic myelogenous leukemia (CML). This haematological malignancy is characterized by the fusion of the BCR gene from chromosome 22 and the ABL gene from chromosome 9 allowing unrestrained BCR-ABL1 associated tyrosine kinase activity, arrested cell differentiation and increased survival of myeloid progenitors. Inhibitors such as imatinib (STI571) and dasatinib directed at the constitutive kinase activity of Bcr/Abl and Src/Abl, respectively restore or activate apoptotic processes in leukaemic blast cells with remarkably therapeutic efficacy in the chronic phase of CML [1]. Unfortunately, the selection/-expansion of BCR-ABL positive mutant cell clones allows CML blast cells become refractory to these drug compounds. Alternative strategies targeting BCR-ABL1 through mechanisms different from those utilized by kinase inhibitors are in clinical trials. For instance, the drug compound FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride) can induce marked apoptosis of CML cells by impairing p210-BCR-ABL activity and expression via the activation of tumour suppressor protein phosphatase PP2A [2]. It appears quite expensive and scarcely reliable to monitor the susceptibility of BCR-ABL and of its mutants to imatinib or dasatinib. Fourier Transform InfraRed microspectroscoy (microFT-IR) has some potential advantages with respect to many traditional techniques that measure molecular components, enzymatic activities or cellular processes, and that are time-consuming and/or have overhead cost. In preliminary experiments we identified early biochemical changes induced in the spectrum of K562 cells by STI571 targeting BCR-ABL (Figure 1). By unsupervised Hierarchical Cluster Analysis (HCA) applied to the region of amides (1690-1480 cm^{-1} wavenumbers interval) we classified the spectra of treated cells in two groups roughly corresponding to the IR spectral profiles of living and of apoptotic cells [3], respectively (Fig. 1a). HCA was also utilized to compare the ability of different drugs to induce cell apoptosis in K562 cells and the percentage of circulating blast cells that were responsive to the treatment in a patient with leukaemia (Fg.1b). Therefore, we aim at developing microFT-IR absorbance spectroscopy as a new readout system for the rapid screening of drugs in “ex vivo” cancer cell models and to identify drug-resistant blast cells in the peripheral blood of patients with leukaemia (Fig. 1c). To implement microFT-IR spectroscopy for drug testing/screening on “ex vivo” cell models, the first objective is to obtain reproducible spectra with acceptable signal-to-noise ratio (S/N) values. They facilitate the identification of IR vibrations reflecting molecular compositions in cells and their changes related to the interference of drugs with specific metabolic pathways [4]. Illuminated areas between 100x100 and 50x50μm^2 at the sample plane represent the actual limits to obtain good quality FT-IR spectra of homogeneous cell populations within few minutes of IR data acquisition when working with Globar and single-channel MCT detector. On the contrary, to identify a single drug-resistant cell among several other drug-sensitive cells, the highest brilliance of SR IR source is required and apt to perform microFT-IR spectroscopy with useful signal from sample areas between 20x20 μm^2 and (sub)cellular scale. In this case IR signals cumulated on the single-channel detector from spot areas fitting the sizes of a cell allow to obtain within few minutes the spectrum of a single cell with acceptable S/N values [5] and to directly compare the spectra of single cells. An interesting development consists in the use of multi-channel IR detector (Focal Plane Array) which, when coupled either a Globar or SR source and -respectively- low (15x) and high (74x) magnification objectives, could allow the IR chemical imaging on either several cell culture areas or single cells within a short acquisition time. The second objective is to cross-validate FT-IR spectra representing specific cellular events by complementary biochemical and morphological analyses conducted in parallel on cells. In the end, the availability of a reference library of cross-validated FT-IR absorbance spectra based on high quality and single cells SR IR analysis will enormously facilitate the identification of spectral profiles and the interpretation of drug-induced spectral variations.

Experimental method. We will apply microFT-IR absorbance spectroscopy on K562 cells, a worldwide “ex vivo” CML cell model utilized to test several drugs. To reduce inter-individual variability, we will synchronize the cell cycle of K562 cells to G0/G1 phase. In time-course experiments, equivalent doses of STI571 and FTY720 drug compounds will be added separately, or in combination, to replicates of logarithmically growing K562 cells. After incubation (20, 60, 180 minutes, respectively), K562 cells and appropriate untreated controls will be fixed for 30 minutes in 1% formalin buffered in PBS, washed with pure water to eliminate PBS, deposited and dried on ZnSe crystals to obtain arrays of sample replicates. Using the visible function of IR microscope we will select single,

isolated cells. IR signals will be acquired in transmission mode from spot areas delimited by apertures fitting the dimensions of a cell (diameter ~10μm); 36x magnification objective/condenser will be used with SR and 100x100μm^2 MCT broadband single detector. In each illuminated sample spot replicates of FT-IR spectra for several individual cells will be acquired in the mid-IR sampling interval from 600 to 4000 cm^{-1} (spectral resolution 4 cm^{-1}) by typically co-adding 256 scans. The complexity of the planned experiment requires a FT-IR spectrometer coupled to IR microscope with fast acquisition capabilities and high spatial resolution. To perform complete characterization of cells, the availability of a dedicated sample preparation area with off line sample preparation equipment nearby the beamline is an asset. Given the quality of IR spectra necessary for their use as reference, SR IR source is justified to obtain the FT-IR absorbance spectra of single cells around 10μm diameter and with usable S/N values within reasonable acquisition time for the experimental statistic significance which this first biomedical diagnostics study aims for. The complementary biochemical information that will be acquired in samples by different analytical techniques will concern de novo protein synthesis, the degree of tyrosine phosphorylation in proteins, and changes occurring at the early stages of apoptosis on plasma membrane (the exposition of phosphatidylserine at the cell surface), and in the nucleus (DNA unwinding and strand breaks).

Safety considerations. Formalin-fixed and air dried cell samples deposited on ZnSe crystal are safe to use (CL1).

Results expected. With a scanner velocity of 20 KHz and an average acquisition time per cell of no less than 2 minutes, roughly 150-200 spectra of single cell will be obtained per shift, taking into account also the time needed to select cells in the sample and to acquire spectra from corresponding backgrounds. Based on previous tests run off line on the B22 end station and conducted in collaboration with G. Cinque (B22 PBS), we expect to achieve absorbance values above 0.2 a.u. within the spectral components of sample acquired in the fingerprint mid-IR region. However, the microFT-IR data acquisition time/set up will be increased/optimized in order to obtain typical N/S<0.05%, and to acquire the maximal number of representative spectra for statistics significance in samples. Some cells with particular shapes, for instance giant cells and shrinking cells, will be mapped at sub-cellular level, for instance in the nucleus, in order to gain major details on intracellular events. Early changes related with the evolution of morphological and biochemical features of cell apoptosis are expected in the spectrum of K562 cells treated with drugs. We also expect that combining biochemical and spectral information in samples we will be able to obtain representative and cross-validated FT-IR absorbance spectra of structural and/or functional events induced in K562 cells by drugs targeting intracellular phosphonetworks.

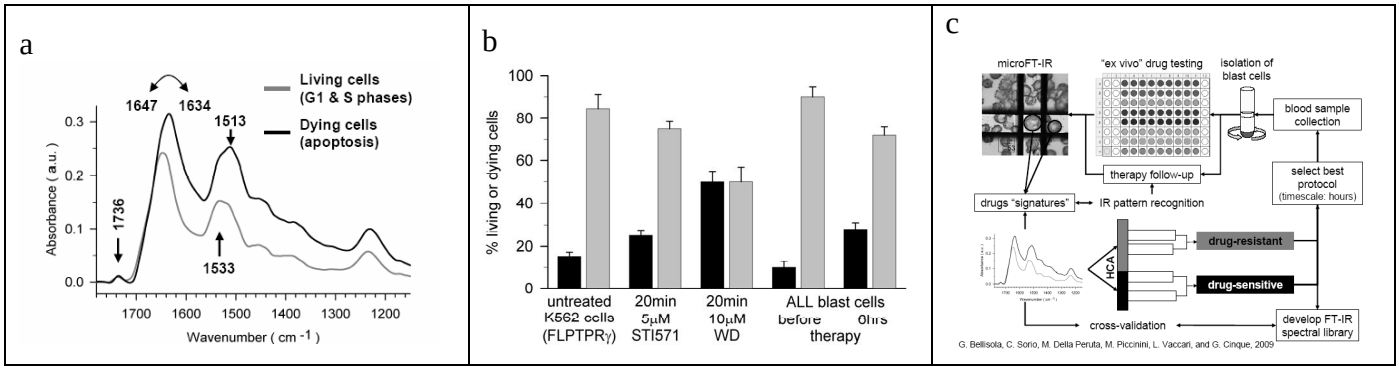

Figure 1: A microFT-IR spectroscopic approach to evaluate drug-resistance/-sensitivity in leukaemic cells. **a**: typical IR markers of apoptosis in the SR FT-IR spectra of single K562 cells. **b**: the evaluation of drug efficacy based on the percentages of living and apoptotic blasts isolated from peripheral blood of a patient with Acute Lymphoblastic Leukaemia (ALL); STI571 = Imatinib mesylate targeting BCR-ABL tyrosine kinase activity; WD: penetratin-conjugated Wedge domain peptide targeting tyrosine phosphatase (PTPRγ). **c**: the flow chart of microFT-IR applications in drug-testing and in the therapy follow-up of leukaemia.

References

[1] S. Padmanabhan, et al. *Future Oncol* **4**, 359-377 (2008).
[2] D. Perrotti, and P. Neviani. *Cancer Metastasis Rev* **27**, 159–168 (2008).
[3] H-Y. N. Holman, et al. *Biopolymers (Biospectroscopy)* **57**, 329–335 (2000).
[4] N. Jamin, et al., *Biopolymers* **72**, 366–373 (2003).
[5] P. Dumas, et al. *Faraday Discuss* **126**, 289-302 (2004).