Tools for cancer research and diagnosis: infrared spectroscopy and microscopy


Since the middle of the 20th century, infrared (IR) spectroscopy coupled to microscopy has been used as a non-destructive, label-free, highly sensitive and specific analytical method to reveal molecular structure. Nowadays, synchrotron based IR microscopy offers a signal-to-noise spectral quality unreachable by other broadband sources, and achieves the highest optical attainable IR spatial resolution on microscopic scale samples. This is particularly relevant in Life Sciences, with a significant progression of applications in biomedical research and in particular cancer studies. In view of the validation of the IR fingerprint region as a spectral marker of cancer and anticancer therapy follow up, we have recently performed a set of key experiments on leukemic blasts at the IR beamline B22 ‘MIRIAM’. The results on identification and cross-validation of IR markers of drug actions in the spectra of K562 leukemic blasts are in the following report.

The underlying principle of IR spectroscopy is that the molecular structure can be revealed by exciting the vibrational modes in materials. The absorption bands in an IR spectrum are thus a fingerprint of the molecular composition. Within the linear range of Lambert-Beer law, the absorbance is a quantitative measure of the molecular species concentration.

Modern IR spectrometers are based on Fourier Transform (FT) interferometer and they are commonly coupled to all-reflective IR microscopes enabling the acquisition of spatially resolved IR information, for instance, in an individual cell. When the microscope aperture defining the IR imaging spot at the sample is reduced to 20 μm or less, the photon throughput towards the detector significantly reduces while the detector noise remains constant, thus the signal to noise ratio (S/N) is strongly decreased. Moreover, when working with microbeams approaching in size the wavelength of mid-IR radiation (above 2.5 to 25 μm wavelength), the diffraction limit becomes dominant in the IR spectral interpretation.

By exploiting Synchrotron Radiation (SR) as an IR source, the brightness of the photon flux density reaching the sample is no more a limitation in the illumination of microscopic sample features. High quality spectra of individual cells with a diameter around 15 μm, which is the average diameter of a graukrohne, were obtained at beamline B22. For reference, samples composed of very homogeneous cell populations were measured and usable spectra with acceptable S/N values were achieved with conventional source from larger areas (e.g. 55μm2), which demonstrated the complementarity of microFT-IR with conventional lab instruments.

The IR spectrum of a cell usually contains a large number of absorption bands, many of them can be confidently assigned to the molecular vibrations of a particular group, in particular analysing the “Fingerprint region”. The unipolar pre-excitation of pre-assigned vibrational modes is not straightforward because the common modes of different molecular components within a cell may overlap and the spectrum may reflect only the average biochemical composition. The development of the so-called cancer fingerprints requires the identification of biomarkers that can be associated to disease-specific molecular pathways. In an attempt to extend the biomedical applications of microFT-IR, we applied mid-IR analysis to search for specific vibrational components with the equivalent significance as biomarkers of the disease. For instance, samples of cancer cells were measured and the analysis of the spectra demonstrated the presence of specific vibrational components that were characteristic of the disease. These results were further validated by applying unsupervised data analysis techniques to the spectra, which allowed the identification of characteristic spectral features that were diagnostic for the disease.

In the attempt to move microFT-IR from spectroscopy labs to hospitals, we have recently demonstrated with a set of experiments performed at the IR beamline B22 ‘MIRIAM’ that a specific region of mid-IR absorbance spectrum is associated to the decrease in tyrosine phosphorylation levels in human leukemic cell models following treatment with the tyrosine kinase inhibitor (TKI). imatinib-mesylate, the founder of a class of highly effective BCR/ABL inhibitors of clinical efficacy. Moreover, we have also identified and cross-validated some typical IR signatures of drug action in cells as shown in Fig. 1.

This scope we acquired SR-based microFT-IR spectrometers of individual leukemic cells exposed to the pro-apoptotic drug imatinib-mesylate (IMA). Typical IR signatures of apoptosis were identified by supervised data analysis in the spectra (Fig. 1A & B). Unsupervised cluster analysis was applied to the dataset allowing the classification of the spectra of cells with apoptotic (drug-sensitive) and viable (drug-resistant) IR patterns (Fig. 1C). This application may suggest that a rapid and economic screening platform based on microFT-IR could be implemented for drug screening of compounds targeting phosphorylation processes or to monitor ex vivo in future, minimal sample treatment, the efficacy of TKI treatment in the individual patients as hypothesised. These qualitative measurements point to a possible application of the technique in clinical trial with patients, where the procedure could be used as a diagnostic tool influencing thereby the treatment of the patients. This could be a step forward for a more efficient and targeted personalized medicine.

References


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