FTIR-spectroscopy for the diagnosis of lung cancer: Can glass be used as a substrate?

By Lewis Dowling, Supervisor: Professor Josep Sulé-Suso
Guy Hilton Research Centre, School of Pharmacy and Bioengineering, Keele University
Email: l.m.m.dowling@keele.ac.uk

Introduction and background

Fourier transform infrared (FITR) spectroscopy is a label-free and non-destructive analytical technique. The spectra produced from FTIR-spectroscopy shows the chemical make up of the sample. This can be used with cells and tissues to provide information on their biochemistry. Spectral bands correspond to the functional groups of the biomolecules in the material (table 1.).

An increasing cancer incidence in the UK is putting greater workload on pathology departments which slows down diagnosis times and impacts patient outcomes. Lung cancer accounts for 13% of cancer cases and has poor survival often due to diagnosis at late stages of the disease [1]. Current diagnostic methods can be time consuming and subjective. FTIR-spectroscopy could form part of an automated system that filters out cancerous samples from non-cancer to improve diagnosis times and patient outcomes.

Wavenumbers (cm^{-1})	Assignment
4000-3100	Dominated by absorption arising from -OH and -NH (amide A) stretching modes.
3100-2800	Absorptions are mainly due to C-H vibrations of CH_2/CH_3 present in protein side chains and hydrocarbon chains of lipids.
1735	Ester C=O stretching in lipids.
1695-1615	Amide I band resulting from C=O stretching in proteins.
1550-1520	Amide II band resulting from N-H bending in proteins.
1200-950	Bands from carbohydrates and phosphate groups in nucleic acids. This region is called the fingerprint region.

Table 1. Vibrations of functional groups in biological materials and their corresponding wavenumbers in FTIR spectra.

Why use glass coverslips as substrates?

Glass is not commonly used as a substrate for FTIR-spectroscopy because it absorbs a portion of the IR radiation causing the fingerprint region be obscured [2]. This removes information on the amide, nucleic acid and carbohydrate portion of biological materials. However traditionally used substrates (CaF_2 and BaF_2) are expensive costing up to £50-60 per slide. This prevents the use of FTIR-spectroscopy in a clinical setting. By using standard glass coverslips of 0.12-0.17mm in thickness the amide I and II bands are visible [3] along with the lipids as shown in fig. 1 below.

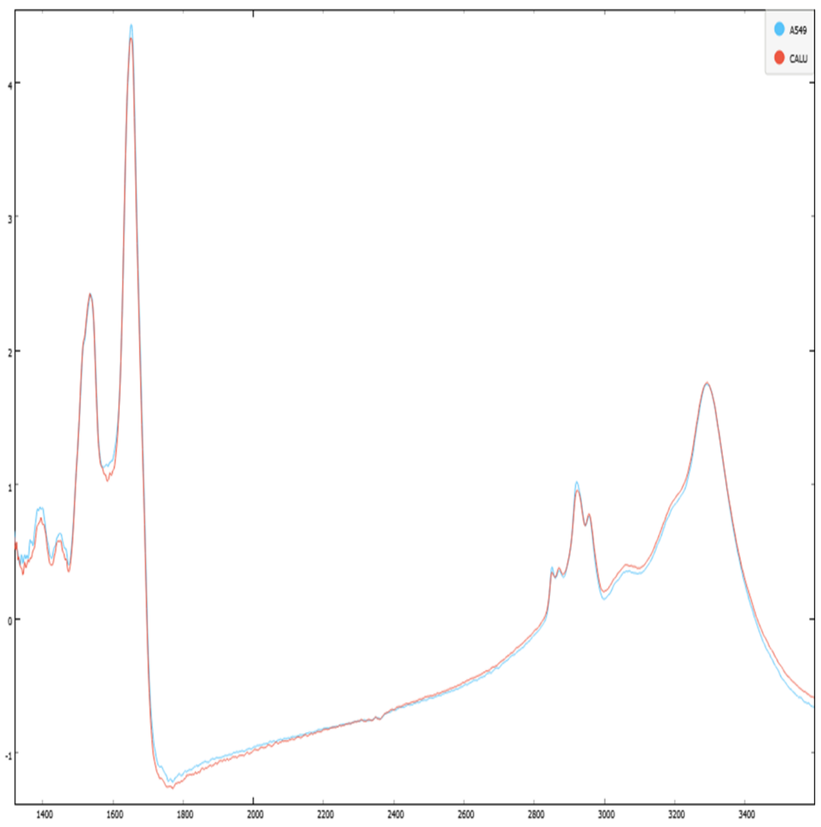

Fig. 1. Mean spectra of A549 and CALU-1 cells on glass coverslips.

Methods

The lung cancer cell lines A549 and CALU-1 were used for this research. Two preparation methods used in pathology laboratories were tested, smears and cytospinning along with two fixatives 4% PFA and methanol. This was done to find the best preparation method while using glass substrates. Spectra of 100 cells of each type taken and averaged. The spectra were de-noised using a Satvisky-Golay filter and normalized using standard normal variate. Spectra were cropped to areas of interest including 3100-2700 cm^{-1} representing lipid bands and 1800-1350 cm^{-1} representing amide I and II bands (see table 1).

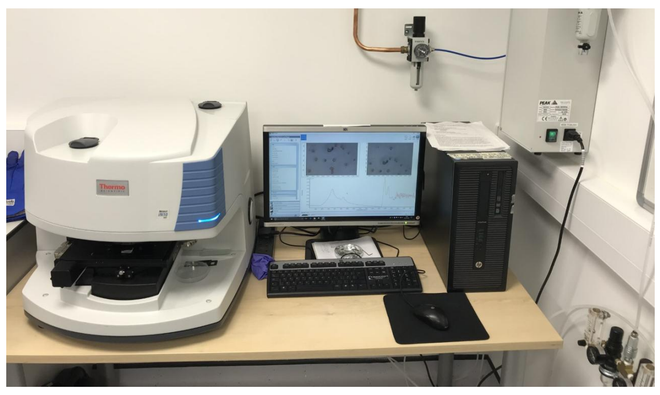

Fig. 2. The Nicolet iN10 FITR-microspectrometer that was used in transmission to conduct the experiments. The instrument contains a globar radiation source and a MCT detector.

References

1. professional/cancer-statistics/incidence#heading-Zero. Published 2018. Accessed August 12, 2020.
2. Rutter A V., Crees J, Wright H, van Pittius DG, Yousef I, Sulé-Suso J. Fourier transform infrared spectra of cells on glass coverslips. A further step in spectral pathology. *Analyst*. 2018;143(23):5711-5717. doi:10.1039/C8AN01634H
3. Rutter A V, Crees J, Wright H, et al. Identification of a Glass Substrate to Study Cells Using Fourier Transform Infrared Spectroscopy: Are We Closer to Spectral Pathology? *Appl Spectrosc*. 2019:3702819875828. doi:10.1177/0003702819875828
4. Dowling L, Roach P, Rutter A V, et al. Optimization of Sample Preparation Using Glass Slides for Spectral Pathology. *Appl Spectrosc*. July 2020:3702820945748. doi:10.1177/0003702820945748

Results

The preparation and fixative methods, cytospins fixed with 4% PFA were chosen as the best option [4]. Methanol removes lipids from the cells therefore a lot of information is lost. Cytospins were chosen as they were more consistent in quality and took less time to analyse due to the cells being concentrated in one area.

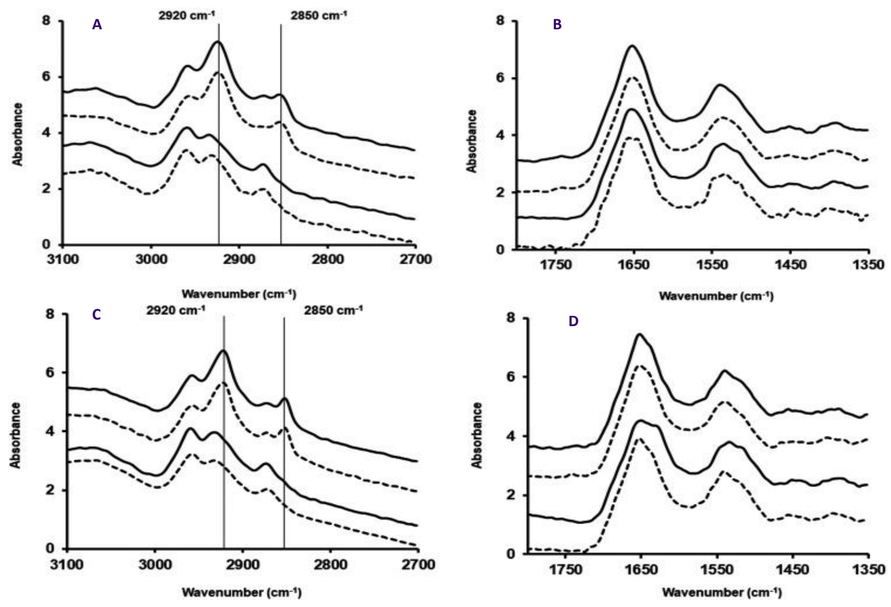

Fig 3. Mean spectra of lipid and Amide regions of CALU-1 (solid lines) and A549 cells (dotted lines) prepared as cytospins (A and B) or smears (C and D) on glass coverslips and fixed with 4% PFA (top two spectra) or methanol (bottom two spectra).

Next steps

The next part of my research will be to investigate identifying normal cells from cancer cells prepared as cytospins and fixed with 4% PFA on glass coverslips. This work will also feature the use of machine learning algorithms to model and predict the cell type from the spectra to demonstrate how the cells could be identified in an automatic system.