PRESENTER
Lewis Dowling

Keele UNIVERSITY

FTIR spectroscopy of lung cells on glass coverslips. A further step towards spectral pathology?

Glass substrates combined with machine learning can be used to classify lung cancer cells from non-malignant lung cells with FTIR spectroscopy.

Background

Cancer incidence rates have been continually rising since the early 1990s [1]. This is putting further pressure on pathology departments which can delay diagnosis and worsen patient outcomes. This has only been aggravated by backlogs from COVID-19. An automated system using Fourier transform infrared (FTIR) spectroscopy could help to improve diagnosis times while providing an objective diagnosis. A major hurdle to the translation of FTIR spectroscopy to a clinical setting is the cost of substrates. Glass is not regularly used as a substrate because the fingerprint region of the spectra is obscured. Using thinner glass allows information on lipids and proteins to be seen [2, 3, 4]. We have shown that enough information is available in the spectra to classify lung cancer cells from non-malignant lung cells using a glass substrate.

Methods

Spectra acquisition

Cell lines
Lung cancer: A549, CALU-1
Non-malignant lung: NL20

Sample preparation
Cells applied to glass coverslip (0.15-0.17mm) by cytospin and fixed with 4% PFA. 3 independent experiments in triplicate.

Measurements
180 spectra of each cell line collected from individual cells using synchrotron transmission FTIR spectroscopy.

→

Pre-processing

Cropping
Spectra cropped to two regions:
3100-2700 cm^{-1} and 1800-1350 cm^{-1}

S-G filter
Window = 21
Polynomial = 2
Derivative = 2

Normalisation
SNV

↓

PCA

Retained 95% of explained variance.

←

Classification

Train/Test
Spectra were randomly sampled. Split 2/3 for training and 1/3 for testing. Repeated 100 times.

KNN
Neighbours = 4
Distance = Euclidean

Random forest
number of trees = 100

Conclusions

- A glass substrate combined with machine learning is viable for classifying lung cancer cells from non-malignant lung cells with FTIR spectroscopy.
- The lipid bands (3100-2700 cm^{-1}) provided the best classification for both A549 and CALU-1 cell lines from NL20 cell line.
- The glass substrates are far more affordable than the CaF_2 and BaF_2 slides currently used.

References

1. 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. 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*. doi:10.1177/0003702820945748

Results

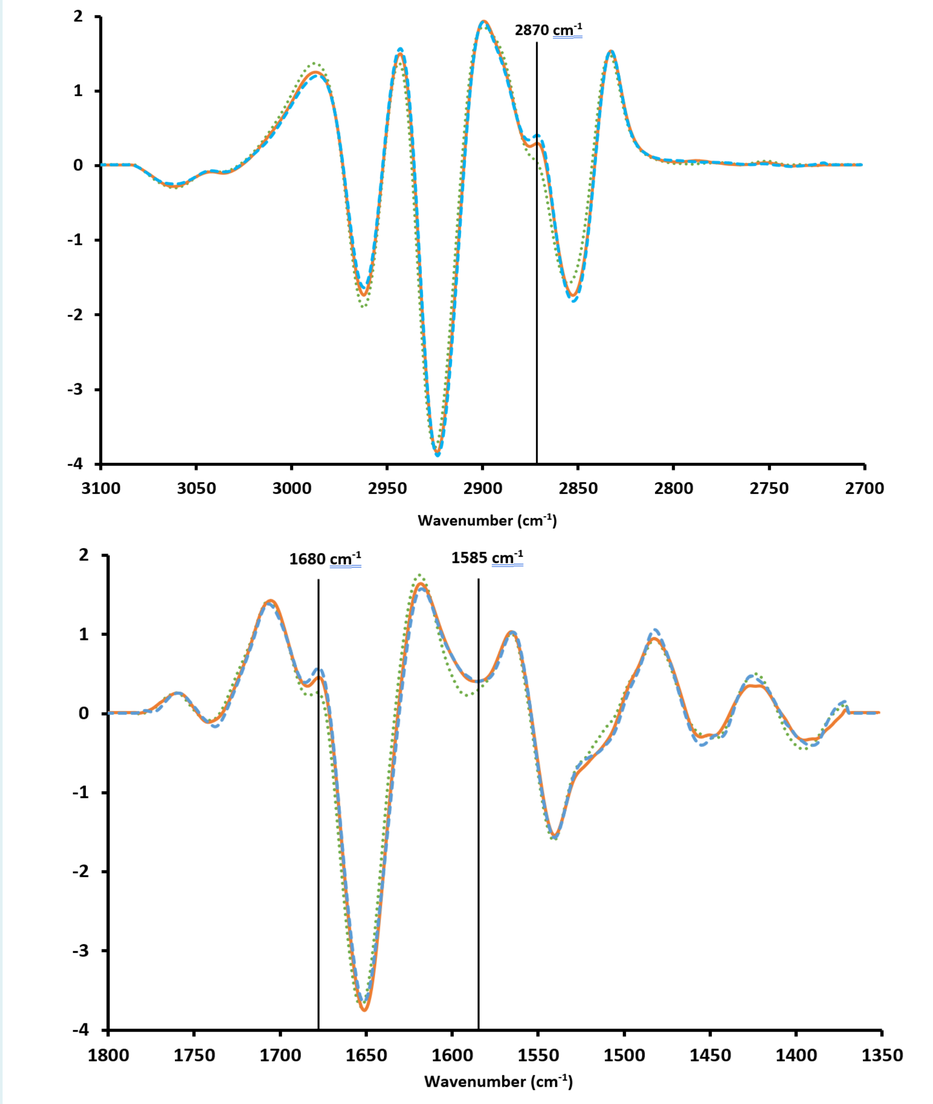

Figure 1. Average 2nd derivative spectra of NL20 (dotted), CALU-1 (dashed), A549 (solid) in the regions 3100-2700 cm^{-1} (top) and 1800-1350 cm^{-1} (bottom).

Cells	Region of spectra	Classifier	AUC	CA	Sensitivity (%)	Specificity (%)
A549 vs NL20	Region between 1800 cm^{-1} to 1350 cm^{-1} (amide I & II)	RF	0.919	0.836	93.2	72.3
		KNN	0.897	0.829	85.1	80.1
	Region between 3100 cm^{-1} to 2700 cm^{-1} (lipids)	RF	0.967	0.916	91.9	91.0
		KNN	0.935	0.891	93.0	84.4
CALU-1 vs NL20	Region between 1800 cm^{-1} to 1350 cm^{-1} (amide I & II)	RF	0.944	0.865	92.5	80.2
		KNN	0.912	0.830	78.0	88.2
	Region between 3100 cm^{-1} to 2700 cm^{-1} (lipids)	RF	0.992	0.953	95.1	94.2
		KNN	0.971	0.940	0.946	0.844

Table 1. Random forest (RF) and K-nearest neighbors (KNN) classification models for A549 vs NL20 and CALU-1 vs NL20 using the spectral regions 3100-2700 cm^{-1} and 1800-1350 cm^{-1}.

[1]DOWLING L, M., [2]YOUSEF I., [3]ROACH P. AND [1,4]SULÉ-SUSO J.
[1]SCHOOL OF PHARMACY AND BIOENGINEERING, KEELE UNIVERSITY, UK; [2]ALBA SYNCHROTRON LIGHT SOURCE, CATALONIA; [3]DEPARTMENT OF CHEMISTRY, LOUGHBOROUGH UNIVERSITY, UK; [1,4]CANCER CENTRE, UNIVERSITY HOSPITALS OF NORTH MIDLANDS, UK