# Raman spectoscopic analysis of exosome induced hypoxia in breast cancer cells

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## Can exosomes induce hypoxia in surrounding breast cancer cells?

To investigate this, exosomes were isolated from triple negative breast cancer cells (cell line MDA-MB-231) grown in normoxia (normal oxygen conditions) and hypoxia (1% oxygen chamber), and these exosomes were then incubated with cells growing in normoxic conditions.

Raman spectroscopy was then used to analyse the biomolecular differences between the cells that have been exposed to normoxic exosomes versus those exposed to hypoxic exosomes, and therefore to determine whether or not exosomes can induce hypoxia in nearby tumour cells.

### What are exosomes?

- Exosomes are microvesicales (40-100 nm in diamter) released by most cells types into the extracellular environment
- Play a significant role in cell signalling and communication, and are therefore linked with the progression of diseases such as cancer [1]
- Are found naturally in all biological fluids

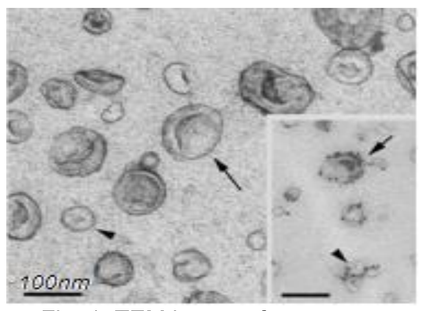


Fig. 1: TEM image of exosomes

### What are hypoxic tumours?

- Hypoxia is a key feature of solid tumours and occurs when the tumour has outgrown its vasculature [2]
- Associated with aggressive tumour behaviour including increased invasiveness and proliferation, the formation of metastasis and poor patient survival rate
- Hypoxic cells are resistant to radiation and chemo therapies [3,4]

### What is Raman spectroscopy?

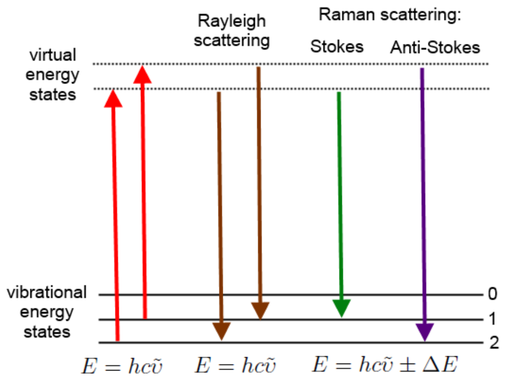


Fig. 2: Energy level diagram comparing Raman and Rayleigh scattering

- Inelastic scattering of laser light
- Non-destructive method
- Minimal sample preparation required
- Weak Raman signal (only 1 in $10^7$ photons) [4]
- High spatial resolution
- Identify the biomolecular composition of samples

## Experimental methods

(1) Cells were grown simultaneously in normoxic and hypoxic chambers
(2) Exosomes were isolated from both batches of cells via centrifugation
(3) An additional two batches of cells were grown in normoxia whilst being subjected to normoxic and hypoxic exosomes respectively
(4) Cells were isolated, formalin fixed and dropped onto a $CaF_2$ slide
(5) The previous steps were repeated three times across different days to minimise the effects of experimental variables

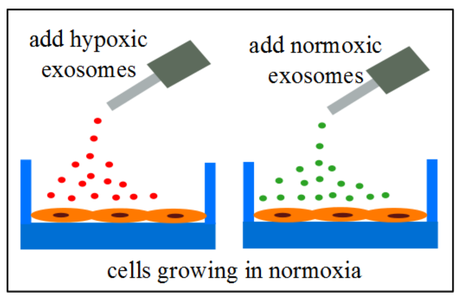


Fig. 3: Illustration of the addition of normoxic/hypoxic exosomes to cells growing in normoxic conditions

Spectroscopic measurement of cells are achieved using a custom Raman system consisting of a laser, detection system (spectrograph and CCD) and a highly efficient optical system to deliver and collect the laser light from the sample with minimum power loss, as shown below:

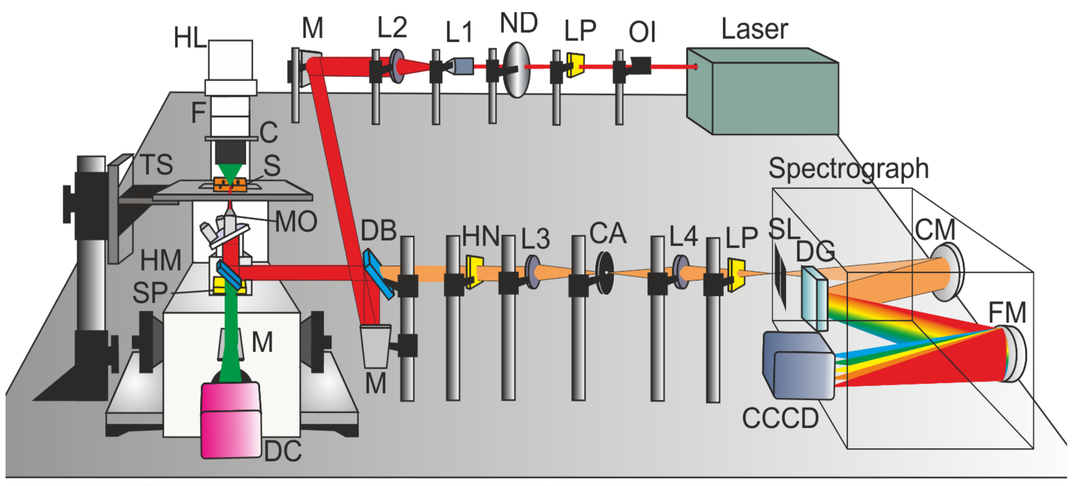


Fig. 4: Schematic of our custom-built Raman spectroscopy set-up

## Results

### Raman spectra:

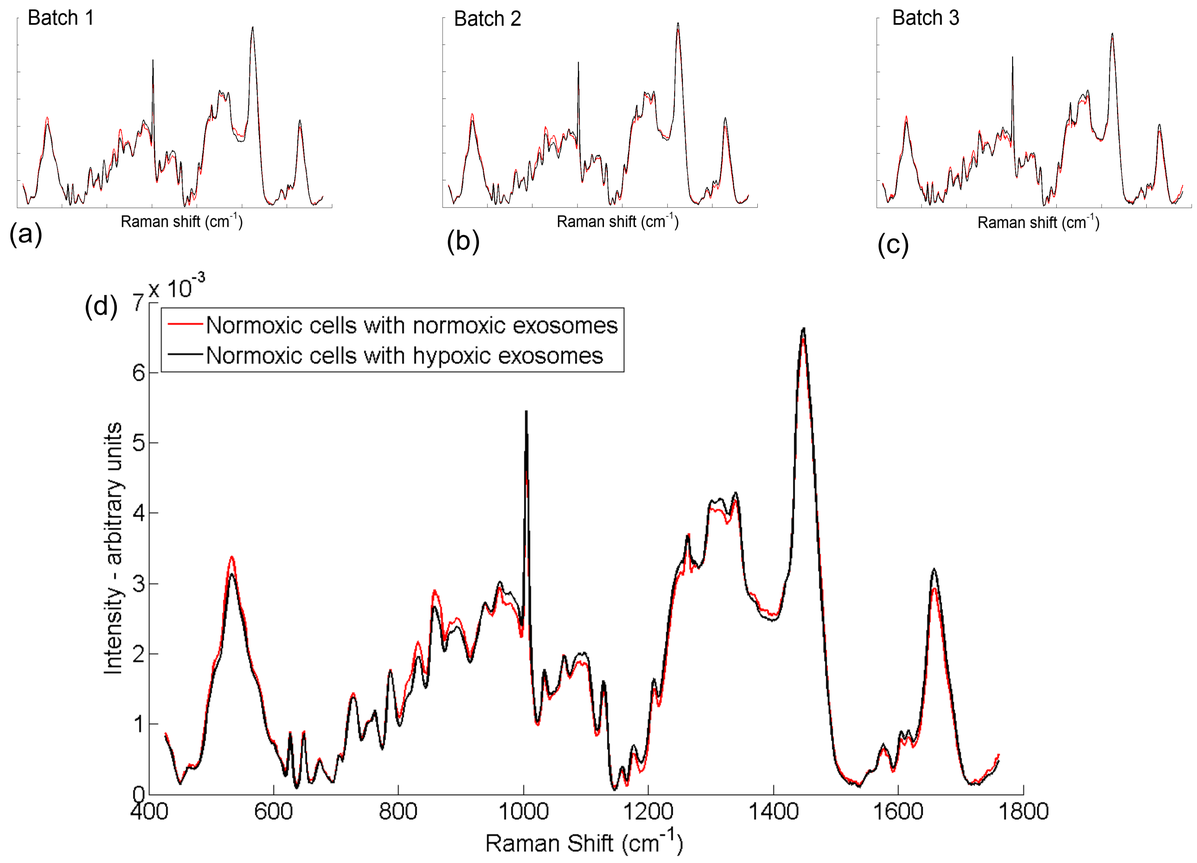


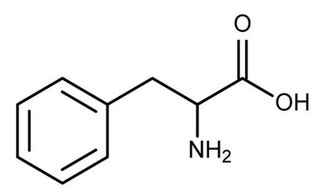
Fig. 5: Raman spectra recorded from normoxic cells grown in the presence of normoxic and hypoxic exosomes, averaged over 50 cells from each, (a) - (c) represent the spectra recorded from each individual batch, and (d) is the overall average from all three batches

### Classification results using PCA and LDA:

| | Sensitivity | Specificity |
|---|---|---|
| Batch 1 | 93.0 % | 95.0 % |
| Batch 2 | 87.2 % | 90.4 % |
| Batch 3 | 76.2 % | 78.0 % |
| All batches combined | 87.4 % | 87.6 % |

### Biomolecular differences:

| Normoxic with normoxic exosomes: | Normoxic with hypoxic exosomes: |
|---|---|
| 829 - O-P-O stretching DNA/RNA<br>Ring breathing tyrosine | 960 - Symmetric stretching vibration of $v_iPO_4^{-3}$ |
| 855 - Proline, tyrosine,<br>Phenylalanine | 1003 - Phenylalanine<br>1088 - C-C stretch<br>1095 - Lipid, phosphodioxy group |
| 890 - Protein bands | 1174 - Tyrosine, phenylalanine<br>1207 - Tryptophan, phenylalanine<br>1337 - Amides, $CH_2/CH_3$ wagging<br>1446 - CH2 benging and deformation<br>1602 - Phenylalanine<br>1615 - Tyrosine, tryptophan<br>1655 - Amide I |




## Conclusion

With this study, we have shown that the biomolecular composition of cells growing in normoxic conditions can change with the presence of hypoxic exosomes. Thus, it can be concluded that exosomes released by nearby hypoxic cells within a tumour can cause normoxic cells to change composition and potentially become hypoxic themselves.

We intend to extend this study to include the composition of normoxic and hypoxic exosomes using Raman spectroscopy and SERS, similar to a previous study [6], in order to investigate the potential use of exosomes as biomarkers for hypoxic tumours.

## References

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