Electronic Supplementary Material (ESI) for Analyst.

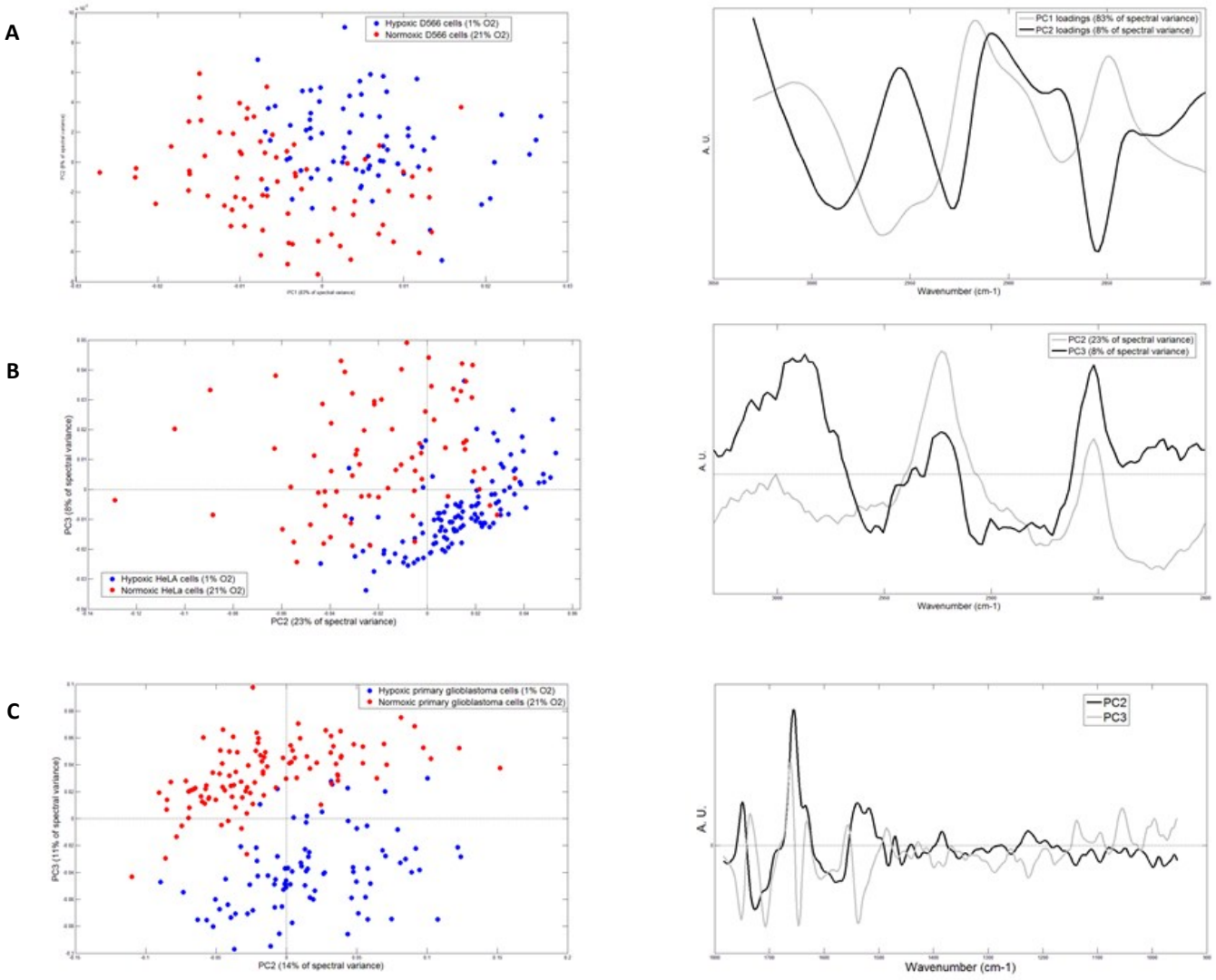
Use Of Infrared Microspectroscopy To Elucidate A Specific Chemical Signature Associated With The Hypoxia Levels Found In Glioblastoma

Christophe Sandt [a*], Céline Nadaradjane, [a,b] Rosalie Richards,[b] Paul Dumas,[a] and Violaine Sée.[b]

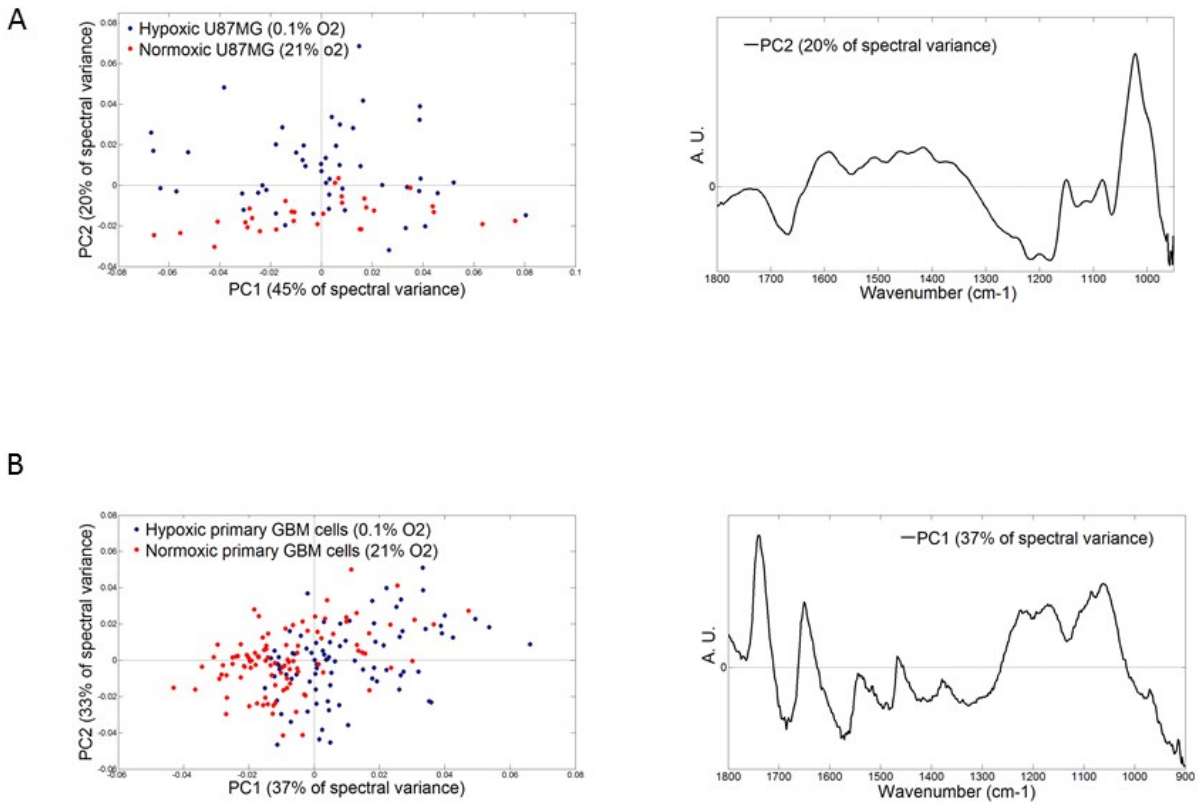
[a] *Synchrotron SOLEIL, L'Orme des Merisiers, 91192 Gif sur Yvette, France*

[b] *Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, UK*

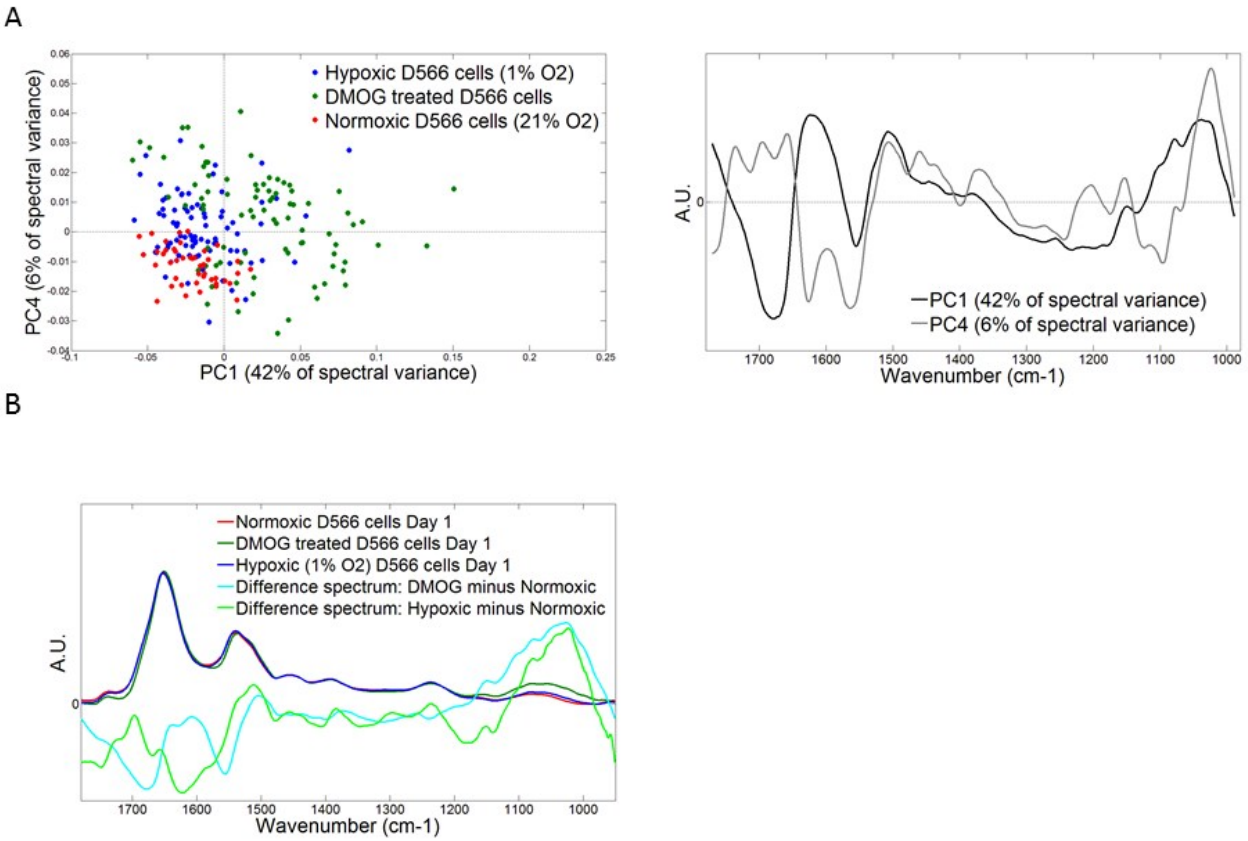
Supplementary figures



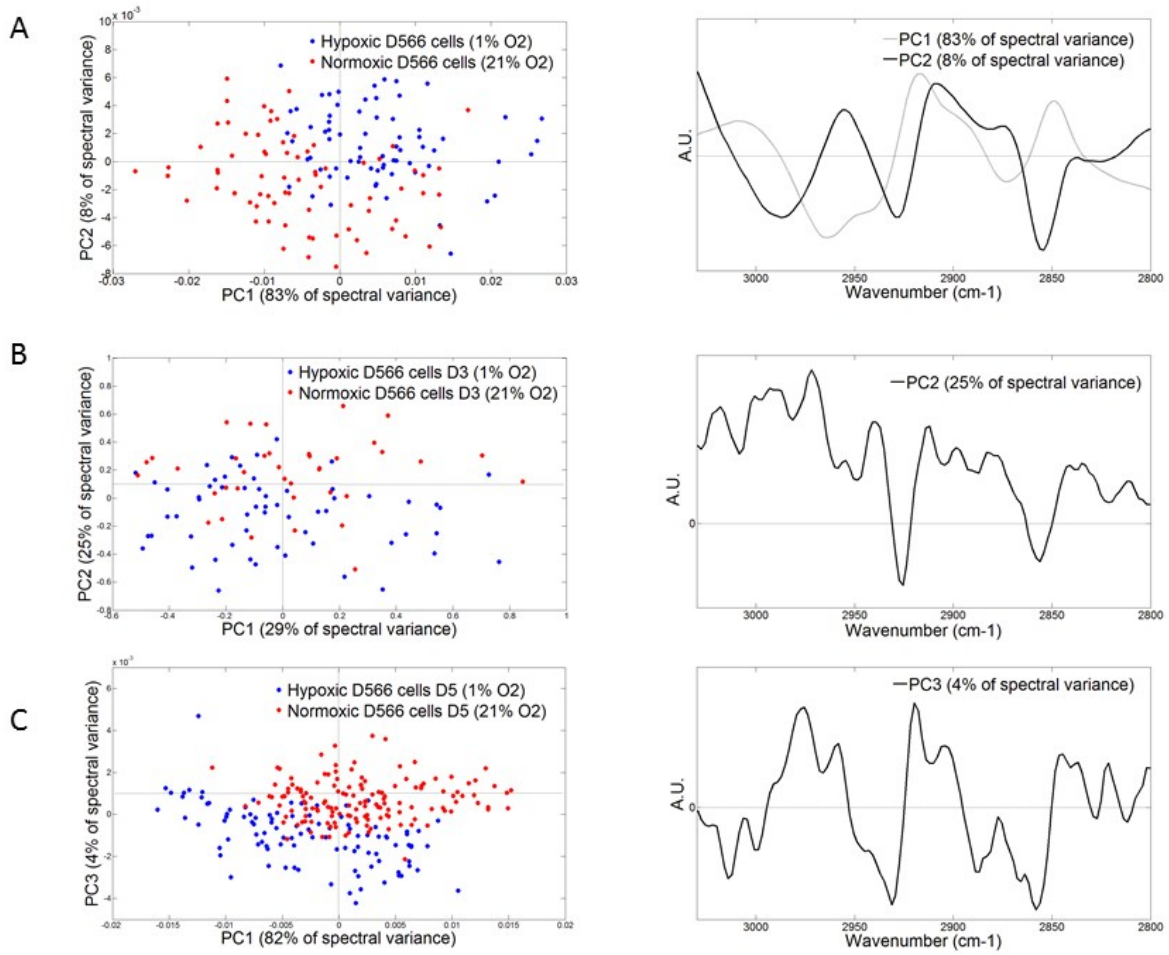
Supplementary figure S1. Detection of hypoxic cells in various cell lines by their infrared spectra. D566 cells, primary GBM cells and HeLa cells exposed to 1% O_2 for 24h were compared to cells grown at 21% O_2. **A)** Comparison of D566 cells exposed to 1% and 21% O_2 by PCA in the CH region (2800-3050 cm^{-1}). The score plot shows the separation between the normoxic and hypoxic cell spectra by a combination of PC1 and PC2. Loadings show an increase of the CH_2 absorption peaks in the hypoxic cells. **B)** Comparison of HeLa cells exposed to 1% and 21% O_2 by PCA in the CH region (2800-3050 cm^{-1}). The score plot shows a separation of hypoxic and normoxic cells by a combination of PC2 and PC3. The loading plot shows an increase in CH_2 absorption peaks in the hypoxic cells. **C)** Comparison of primary GBM cells exposed to 1% and 21% O_2 by PCA in the fingerprint region (900-1800 cm^{-1}). The score plot shows a separation of hypoxic and normoxic cells by PC3. The loading plot shows an increase of lipid absorption peaks at 1740, 1460, 1380 cm^{-1} and changes in the protein signal between 1480 and 1700 cm^{-1}.



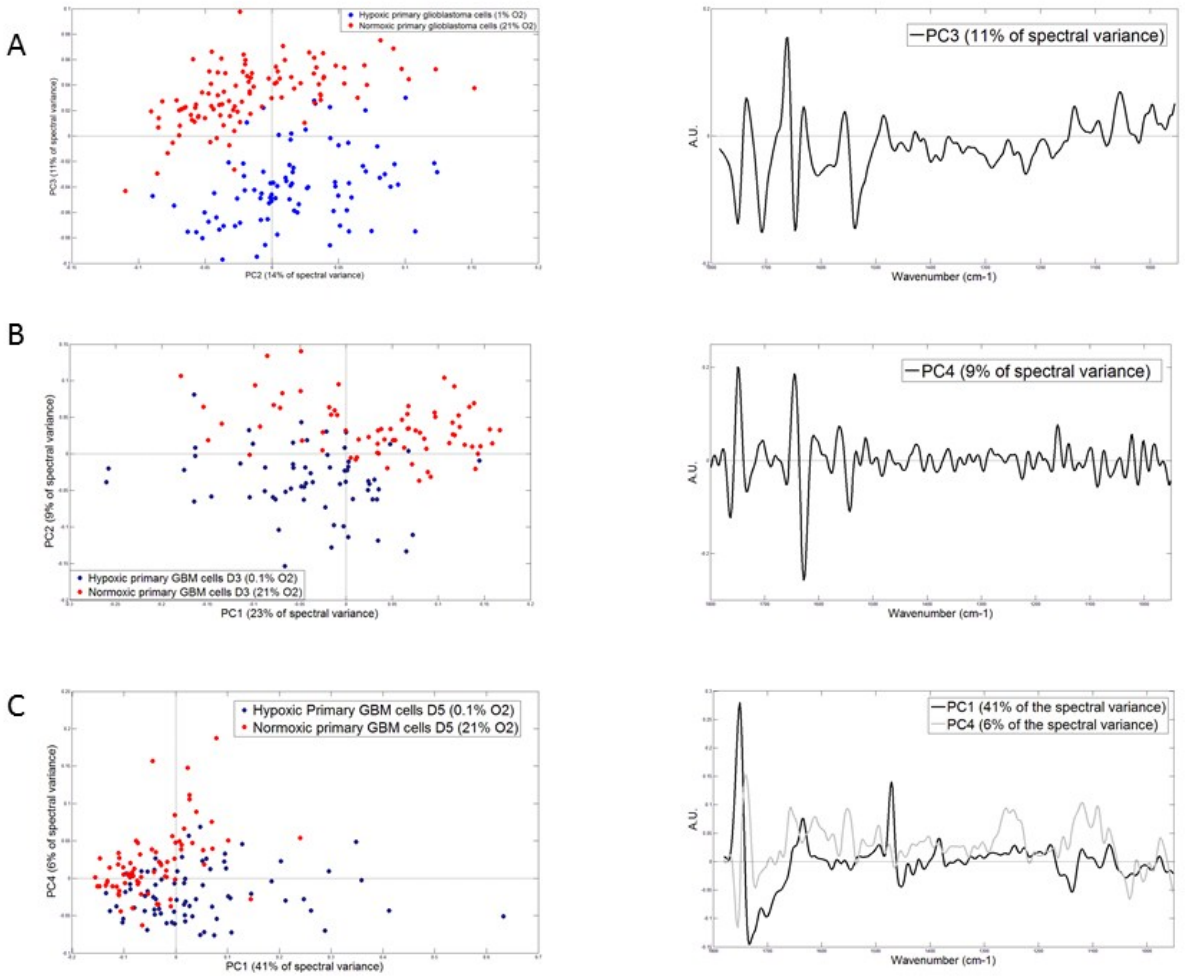
Supplementary figure S2. Spectral signatures of U87MG and primary GBM cells cultured at 0.1% O_2. A) Comparison of U87MG cells exposed to 0.1 and 21% O_2 for 24h by PCA in the fingerprint region. The score plot shows that hypoxic and normoxic cells are separated by PC2. The loading plot shows that PC2 codes for an increase in glycogen in the hypoxic cells. **B)** Comparison of primary GBM cells exposed to 0.1 and 21% O_2 for 24h by PCA in the fingerprint region. The score plot shows a partial separation between hypoxic and normoxic cells along the PC1 axis. The loading plot shows that PC1 codes for increases in lipids, polysaccharides and proteins in the hypoxic cells.



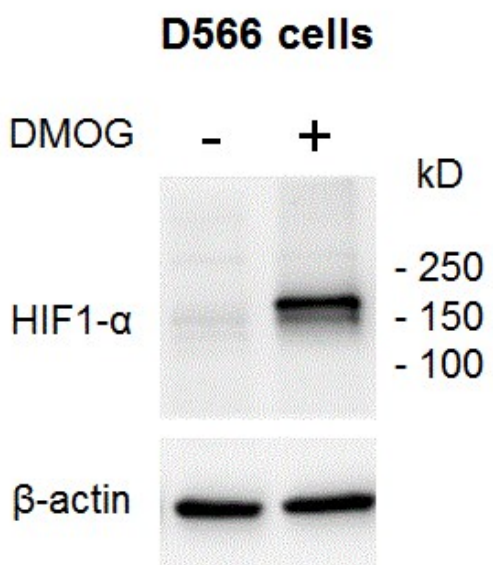
Supplementary figure S3. Effects of DMOG or 1% O_2 in D566 cells. A) Comparison of the spectra from normoxic, hypoxic and DMOG treated cells after 24h culture by PCA. The score plot shows that DMOG-treated and hypoxic cells are clustered apart from normoxic cells by a combination of PC1 and PC4. DMOG-treated are clustered further apart from normoxic cells evidencing a stronger effect of DMOG. The PCA loading plot shows that PC1 and PC4 codes for changes in the protein and polysaccharide signals. **B)** Comparison of normoxic, hypoxic and DMOG treated cells by differential spectroscopy. Difference spectra show that DMOG treated cell spectra exhibit similar but stronger changes than hypoxic cells in the polysaccharide region (950-1200 cm^{-1}), confirming the PCA result.



Supplementary figure S4. Stability of the hypoxic signature over time in D566 cells. Comparison of normoxic and hypoxic (1% O_2) cell spectra at day 1, day 3 and day 5 by PCA in the CH region (D566). PCA score plot and loading plot of D566 cell spectra at day 1 (A), day 3 (B) and day 5 (C) showing a separation between normoxic and hypoxic cells and an increase in lipid signal in hypoxic cells. The percentage of variance between hypoxic and normoxic cells decreases from around 30% at day 1 and day 3 to 4% at day 5.



Supplementary figure S5. Stability of the hypoxic signature over time in primary GBM cells. Comparison of normoxic and hypoxic (0.1% O_2) cell spectra at day 1, day 3 and day 5 by PCA in the fingerprint region. PCA score plot and loading plot of primary GBM cells show changes in lipid, protein and carbohydrate signals. **A)** Separation between hypoxic and normoxic cells at day 1 is by PC3 coding for 11% of the variance. **B)** Separation at day 3 by PC4 coding for 9% of the spectral variance. **C)** Separation at day 5 is carried mostly by PC4 coding for 6% of the variance.



Supplementary figure S6. Up-regulation of HIF1 α expression upon DMOG treatment in D566 cells. HIF-1α levels were measured by western-blot in D566 cells either non-treated or treated with 0.5mM DMOG for 6h. Control for equal loading is shown with beta-actin levels. Western-blot methodology and the antibodies used were previously described in Herrmann et al., 2015[4].