



# Survival of patients with advanced metastatic melanoma: the impact of novel therapies—update 2017

**DOI:**

[10.1016/j.ejca.2017.06.028](https://doi.org/10.1016/j.ejca.2017.06.028)  
[10.1016/j.ejca.2017.06.028](https://doi.org/10.1016/j.ejca.2017.06.028)

**Document Version**

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

**Citation for published version (APA):**

Ugurel, S., Röhmel, J., Ascierto, P. A., Flaherty, K. T., Grob, J. J., Hauschild, A., Larkin, J., Long, G. V., Lorigan, P., McArthur, G. A., Ribas, A., Robert, C., Schadendorf, D., & Garbe, C. (2017). Survival of patients with advanced metastatic melanoma: the impact of novel therapies—update 2017. *European Journal of Cancer*, Article PMID 28756137. <https://doi.org/10.1016/j.ejca.2017.06.028>, <https://doi.org/10.1016/j.ejca.2017.06.028>

**Published in:**

European Journal of Cancer

**Citing this paper**

Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

**General rights**

Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

**Takedown policy**

If you believe that this document breaches copyright please refer to the University of Manchester's Takedown Procedures [<http://man.ac.uk/04Y6Bo>] or contact [openresearch@manchester.ac.uk](mailto:openresearch@manchester.ac.uk) providing relevant details, so we can investigate your claim.





## Raman Spectroscopy as a Novel Method in Placental Research: Recognizing the Pattern of Placental Hypoxia

Journal:	<i>Journal of Raman Spectroscopy</i>
Manuscript ID	JRS-17-0084.R2
Wiley - Manuscript type:	Short Communication
Date Submitted by the Author:	n/a
Complete List of Authors:	Schlabritz-Loutsevitch, Natalia; Texas Tech University Health Sciences Center at the Permian Basin, Obstetrics and Gynecology Gandhi, Kushal; Texas Tech University Health Sciences Center at the Permian Basin, Obstetrics and Gynecology Soydemir, Fatimah; University of Manchester, Maternal and Fetal Medicine Health Research Centre Brownbill, Paul; University of Manchester, Maternal and Fetal Health Research Centre Sengar, Raghvendra; Methrohm USA, Spectroscopy Sales Ventolini, Gary; Texas Tech University Health Sciences Center at the Permian Basin, Obstetrics and Gynecology Bruillard, Paul; Pacific Northwest National Laboratory, Computational Mathematics Gosink, Luke; Pacific Northwest National Laboratory, Computational Mathematics
Keywords:	Raman Spectroscopy, Placenta, hypoxia, pattern recognition

SCHOLARONE™  
Manuscripts

1

1

**SHORT COMMUNICATION****RAMAN SPECTROSCOPY AS A NOVEL METHOD IN PLACENTAL RESEARCH:  
RECOGNIZING THE PATTERN OF PLACENTAL HYPOXIA**

Natalia Schlabritz-Loutsevitch<sup>1\*</sup>, Kushal Gandhi<sup>1</sup>, Fatimah Soydemir<sup>2,3</sup>, Paul Brownbill<sup>2,3</sup>,  
Raghvendra Sengar<sup>4</sup>, Gary Ventolini<sup>1</sup>, Paul Bruillard<sup>5</sup> and Luke Gosink<sup>5</sup>.

<sup>1</sup>Texas Tech University Health Sciences Center at the PB, Odessa, TX, USA

<sup>2</sup>Maternal and Fetal Health Research Centre, School of Medical Sciences, Faculty of Biology,  
Medicine & Health, University of Manchester, St. Mary's Hospital, Oxford Road, Manchester,  
M13 9WL UK

<sup>3</sup>Maternal and Fetal Health Research Centre, St. Mary's Hospital, Central Manchester University  
Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester  
M13 9WL, UK

<sup>4</sup>Metrohm, USA

<sup>5</sup>Pacific Northwest National Laboratory, Richland, WA, USA

**\*Corresponding author**

Natalia Schlabritz-Lutsevich MD, PhD  
Research Associate Professor (tenure-track)  
Department of Obstetrics and Gynecology  
Department of Pharmacology and Neuroscience  
Texas Tech University HSC School of Medicine  
e-mail: Natalia.schlabritz-lutsevich@ttuhsc.edu  
office phone: 432-703-5169  
cell phone: 210-317-0156  
fax: 432-335-5140

2

**ABSTRACT**

Raman spectroscopy (RS) is a non-elastic photon scattering technique. We evaluated whether a specific RS pattern exists in fetal venous perfusates obtained at 30-60 min intervals from the *ex vivo* human dual placental perfusion model under hypoxic (n=6) and normoxic (n=5) conditions. A stratified Principle Component and a linear support vector machine analyses showed that the separation between two conditions was readily feasible at 60-120 min of perfusion; after this any apparent differences were most likely the result of artifact over-fitting. This report is the first attempt to identify the RS fingerprint associated with placental hypoxia.

**Keywords:** Raman spectroscopy, placenta, hypoxia, pattern recognition.

## INTRODUCTION

In the 21<sup>st</sup> century diagnostic methods expanded beyond the identification of single molecular species<sup>[1] [2]</sup>. Raman Spectroscopy (RS), a method based on inelastic light scattering when excitation photons interfere with specific molecular bonds<sup>[3]</sup>, represents a unique tool currently being applied to the diagnosis of cancer, Alzheimer and cardio-vascular diseases<sup>[4] [5]</sup>. **Resonance RS and Raman microspectroscopy have been applied for detection of hemo-and myo-globin oxygenation for monitoring of tissue oxygenation<sup>[6] [6-7]</sup> and for molecular fingerprints of pre-eclamptic placentas<sup>[8]</sup>. Orbital Raster Scanning (ORS) is a novel RS sampling technique based on rastering laser beam, which repetitively sweeps the sample volume providing the opportunity to represent thousands of chemical compounds simultaneously within a spectral “fingerprint”. The rastering approach allows scan the larger area, e.g. while the focused laser beam, has a diameter of 50 $\mu$ m, the raster spot diameter is approximately 1 mm. This gives an ORS sampling area of approximately 0.076 mm<sup>2</sup> versus an instantaneous sampling area of 0.0078 mm<sup>2</sup>.<sup>[9]</sup> The application of mathematical models of pattern recognition enables an analysis between experimental study groups. Here we demonstrate, for the first time, the application of RS-ORS to detect fetal hypoxia in physiological perfusates following the adaptation of the *ex vivo* dual perfused human placenta to different oxygenation conditions.**

## MATERIALS AND METHODS

We analyzed 500  $\mu$ L of fetal perfusate, obtained using the human *ex vivo* dual placental perfusion technique, which had been modified to normoxic (N; n=5) and hypoxic (H; n=6) conditions, with mean soluble oxygen tension within the intervillous space (IVS) of 5-7% and < 3%, respectively<sup>[10]</sup>. These adaptations have been proven to have specific changes in

4

1  
2  
3  
4 80 inflammatory mediation within the perfused tissue, demonstrated as an altered biochemical  
5  
6 81 release of cytokines <sup>[10a]</sup>. The perfusate (modified Earle' bicarbonate buffer) composition was as  
7  
8 82 follows 2.4 mM CaCl<sub>2</sub>; 0.4mM MgSO<sub>4</sub>; 117 mM NaCl; 5.4mM KCl; 0.41mM NaH<sub>2</sub>PO<sub>4</sub>;  
9  
10 83 26mM NaHCO<sub>3</sub>; 5.6mM D-glucose; 5,000 IU heparin, sodium; 0.04mM L-arginine; 0.1% (w/v)  
11  
12 84 bovine serum albumin (fraction IV); 3.5% (w/v) dextran (clinical grade; 70KDa)<sup>[10b]</sup>. Fetal  
13  
14  
15 85 venous perfusate samples were collected at 60, 120, 150, 210, 240, 300, and 330 minutes  
16  
17 86 (Supplementary material, Fig. 1S). The RS of the samples were collected using Mira Cal  
18  
19 87 software (Mira M-1, Metrohm, USA), which was connected to the hand-held RS device (Mira  
20  
21 88 M-1, Metrohm, USA). In MIRA M1 device, exposure time is automated by the analyzer, the  
22  
23 89 exposure time was 5 second with spectral resolution: 14 to 16 cm<sup>-1</sup>.  
24  
25  
26  
27

28 90 **Insert Fig. 1 here**

29  
30  
31 91 For the Principal component analyses the spectral analysis began by constructing a tuple of  
32  
33 92 information from the twelve fingerprint regions, identified by picking maximum peak heights for  
34  
35 93 peaks that were greater than the mean value of all spectra values (Fig 1). For each of these  
36  
37 94 regions, a feature vector was constructed based on three peak attributes: the peak's value (x), the  
38  
39 95 derivative for the peak (x'), and the second derivative for the peak (x''). Thus for a given time  
40  
41 96 (min) and class (i.e., H vs. N), a 36 dimensional feature vector was constructed (i.e. 12 peaks  
42  
43 97 times, 3 attributes per peak).  
44  
45  
46  
47

48 98 A stratified Principle Component Analysis (PCA) <sup>[11]</sup> was performed independently on the  
49  
50 99 feature vector data aggregated at each time point. For each time point, a set of distinct principle  
51  
52 100 components were identified where each subsequent set of components describes an increasing  
53  
54 101 amount of variance in the feature vector data: e.g., 50%, 60%, ..., 100%. A linear support vector  
55  
56  
57  
58  
59  
60

5

1  
2  
3 102 machine (SVM) <sup>[12]</sup> was then used to compute the accuracy of each component set. In this  
4  
5 103 context, the analysis contrasts how increasing information content (as represented by different  
6  
7  
8 104 principle components) impacts classification performance.  
9  
10  
11 105

## 106 RESULTS AND DISCUSSION

107 Twelve Raman peaks were detected at 415 cm<sup>-1</sup>, 440 cm<sup>-1</sup>, 541 cm<sup>-1</sup>, 785 cm<sup>-1</sup>, 851 cm<sup>-1</sup>, 920 cm<sup>-1</sup>,  
108 1, 1020 cm<sup>-1</sup>, 1086 cm<sup>-1</sup>, 1129 cm<sup>-1</sup>, 1341 cm<sup>-1</sup>, 1457 cm<sup>-1</sup>, 1632 cm<sup>-1</sup> (Fig.1S). The hypoxic  
109 treatment of placenta *ex vivo* in our study represents the models, mimicking pre-eclampsia (PE)  
110 <sup>[10a]</sup>, interestingly, these spectra are similar to the Raman shift, reported for human normal and  
111 pre-eclamptic placentas <sup>[8]</sup> and blood samples from women with pre-eclampsia<sup>[13]</sup>. The Raman  
112 peak of 1129cm<sup>-1</sup> was quite close to 1127 cm<sup>-1</sup> hemoglobin-derived hemoporphirin shift reported  
113 in exposed to hypoxia erythrocytes and myocardium<sup>[6, 14]</sup> and the same as found in placentas  
114 from women with pre-eclampsia <sup>[8]</sup>. 1128 cm<sup>-1</sup> Raman shift was identified for myristic acid,  
115 which has also chain vibration expansion shift of 414 cm<sup>-1</sup><sup>[15]</sup> – close to detected in our study 415  
116 cm<sup>-1</sup>. Band at the shift of 1341 cm<sup>-1</sup> had decreased intensity in samples with hypoxia in our study  
117 and is similar to reported 1342 cm<sup>-1</sup> shift with decreased intensity in serum of women with PE,  
118 which has been identified as CH bending of amino acids<sup>[13]</sup>. The 1457 cm<sup>-1</sup> shift is located within  
119 1420-1500 cm<sup>-1</sup> region of described deformities for  $\delta$  (CH<sub>2</sub>) and  $\delta$  (CH<sub>3</sub>) region of L-arginine<sup>[15]</sup>.  
120 The peak of 440 cm<sup>-1</sup> corresponds to  $\beta$ -D-glucose and peak at 541 cm<sup>-1</sup> -to D (+) dextrose <sup>[15]</sup> –  
121 components of the perfusion media. At the 60 min time point the separation between N and H  
122 (Figure 2A) is easily obtained with 100% accuracy using principle components that capture 80%

123 of the variability in the feature vector data. Even when using fewer features where 50% of the  
124 variability is captured, separation is feasible with 80% accuracy.

125 **Insert Fig. 2 here**

126 At 120 minutes (Figure 2B), a slight degradation in accuracy is observed. Specifically, we now  
127 need almost 88% of the variance captured by the principle components to provide 100%  
128 accuracy in separating two conditions (as opposed to 80% seen in 60 minutes). Additionally,  
129 classifying based on PCA that captures 50% of the variance only provides about 75% accuracy.

130 Beyond 120 minutes (Figure 2C and D), significant degradation of the differential signal is  
131 observed. Though 100% classification accuracy is feasible (i.e. using principle components that  
132 capture *all* variance in data), removing even a little of the information in the data (i.e. from 95%  
133 to 100%) dramatically reduces accuracy. Such behavior strongly indicates that beyond 120  
134 minutes of perfusion, separation between two conditions is an artifact of overfitting or could  
135 mirror the phenomenon of slow progression of normoxia toward hypoxia in the artificial *ex vivo*  
136 perfusion system <sup>[10b]</sup>. To more confidently address this artifact and confirm the ability (or  
137 inability) to separate data beyond 120 minutes, an additional number of samples is required.  
138 Commensurate with a reduction in classification accuracy after the 120 minute time point  
139 between normoxic and hypoxic groups, the fetal-side release profile of endogenous placental  
140 substances appears to be strongly governed by intervillous space soluble oxygenation within this  
141 timeframe, since the difference in this variable is highest within the first two hours and  
142 diminishes thereafter <sup>[10b]</sup>. Raman spectra are produced by the portion of photons, scattered by  
143 electron density of specific molecular bonds <sup>[5a]</sup> and depend on vibrational activity of the bonds.  
144 Therefore, it is also possible that the **intervillous space** hypoxia results in the differences in



7

1  
2  
3 145 vibrational activity of the bonds, prior to the release of any active substances in the fetal  
4  
5  
6 146 circulation and therefore could be a sensitive method of diagnosis of fetal hypoxia.  
7  
8

## 9 147 **ACKNOWLEDGEMENTS**

10  
11 148 We are indebted to the Department of Obstetrics and Gynecology (Department Chair Dr. Moss  
12  
13 149 Hampton) for the continuous support.

## 14 150 **FIGURE LEGENDS**

15  
16  
17 151 **Figure 1.** Example of representative peaks used for the vector construction.

18  
19 152 **Figure 2.** The accuracy of a linear support vector machine to separate hypoxia from normoxia at  
20  
21 153 60, 120, 150 and 210 min of the placental perfusion *ex vivo*.

154

## 22 23 24 25 155 **SUPPLEMENTARY MATERIAL**

26  
27 156 **Figure 1S.** Mean Raman Spectroscopy patterns in the fetal perfusates obtained at 30-60 min  
28  
29 157 intervals in *ex vivo* placental perfusion: hypoxic (n=6) and normoxic (n=5) conditions <sup>[10b]</sup>.

158

## 30 31 32 33 159 **REFERENCES**

- 34  
35 160 [1] F. Mousavi, B. Bojko and J. Pawliszyn, *Anal Chim Acta* **2015**, *892*, 95-104.  
36 161 [2] J. Peng, F. Tang, R. Zhou, X. Xie, S. Li, F. Xie, P. Yu and L. Mu, *Acta Pharm Sin B* **2016**, *6*, 540-551.  
37 162 [3] M. Jermyn, J. Desroches, K. Aubertin, K. St-Arnaud, W. J. Madore, E. De Montigny, M. C. Guiot, D.  
38 163 Trudel, B. C. Wilson, K. Petrecca and F. Leblond, *Phys Med Biol* **2016**, *61*, R370-r400.  
39 164 [4] K. Kong, C. Kendall, N. Stone and I. Notingher, *Adv Drug Deliv Rev* **2015**, *89*, 121-134.  
40 165 [5] a) I. Pence and A. Mahadevan-Jansen, *Chem Soc Rev* **2016**, *45*, 1958-1979; b) C. R. Kong, I. Barman, N.  
41 166 C. Dingari, J. W. Kang, L. Galindo, R. R. Dasari and M. S. Feld, *AIP Adv* **2011**, *1*, 32175.  
42 167 [6] A. Almohammed, S. M. Kapetanaki, B. R. Wood, E. L. Raven, N. M. Storey and A. J. Hudson, *J R Soc*  
43 168 *Interface* **2015**, *12*.  
44 169 [7] a) K. R. Ward, I. Torres Filho, R. W. Barbee, L. Torres, M. H. Tiba, P. S. Reynolds, R. N. Pittman, R. R.  
45 170 Ivatury and J. Turner, *Crit Care Med* **2006**, *34*, 792-799; b) I. P. Torres Filho, J. Turner, R. N. Pittman, E.  
46 171 Proffitt and K. R. Ward, *J Appl Physiol (1985)* **2008**, *104*, 1809-1817.  
47 172 [8] S. J. Chen, Y. Zhang, X. P. Ye, K. Hu, M. F. Zhu, Y. Y. Huang, M. Zhong and Z. F. Zhuang, *Arch Gynecol*  
48 173 *Obstet* **2014**, *290*, 943-946.  
49 174 [9] P. A. Mosier-Boss and M. D. Putnam, *Anal Chem Insights* **2013**, *8*, 83-97.  
50 175 [10] a) A. Jain, H. Schneider, E. Aliyev, F. Soydemir, M. Baumann, D. Surbek, M. Hediger, P. Brownbill and  
51 176 C. Albrecht, *Lab Invest* **2014**, *94*, 873-880; b) F. Soydemir, S. Kuruvilla, M. Brown, W. Dunn, P. Day, I. P.  
52 177 Crocker, P. N. Baker, C. P. Sibley and P. Brownbill, *Lab Invest* **2011**, *91*, 181-189.  
53 178 [11] H. Abdi, Williams, L.J. , *Wiley Interdisciplinary Reviews: Computational Statistics* **2010**, *2*, 433-459.  
54 179 [12] V. V. Corinna Cortes, *Machine Learning* **1995**, *20*, 273-297.

8

1  
2  
3 180 [13] U. P. Günay Başar, Şeyma Şeninak, Tuba Günel, Ali Benian, and İbrahim Kalelioğlu *Spectroscopy: An*  
4 181 *International Journal* **2012**, 27, 14.

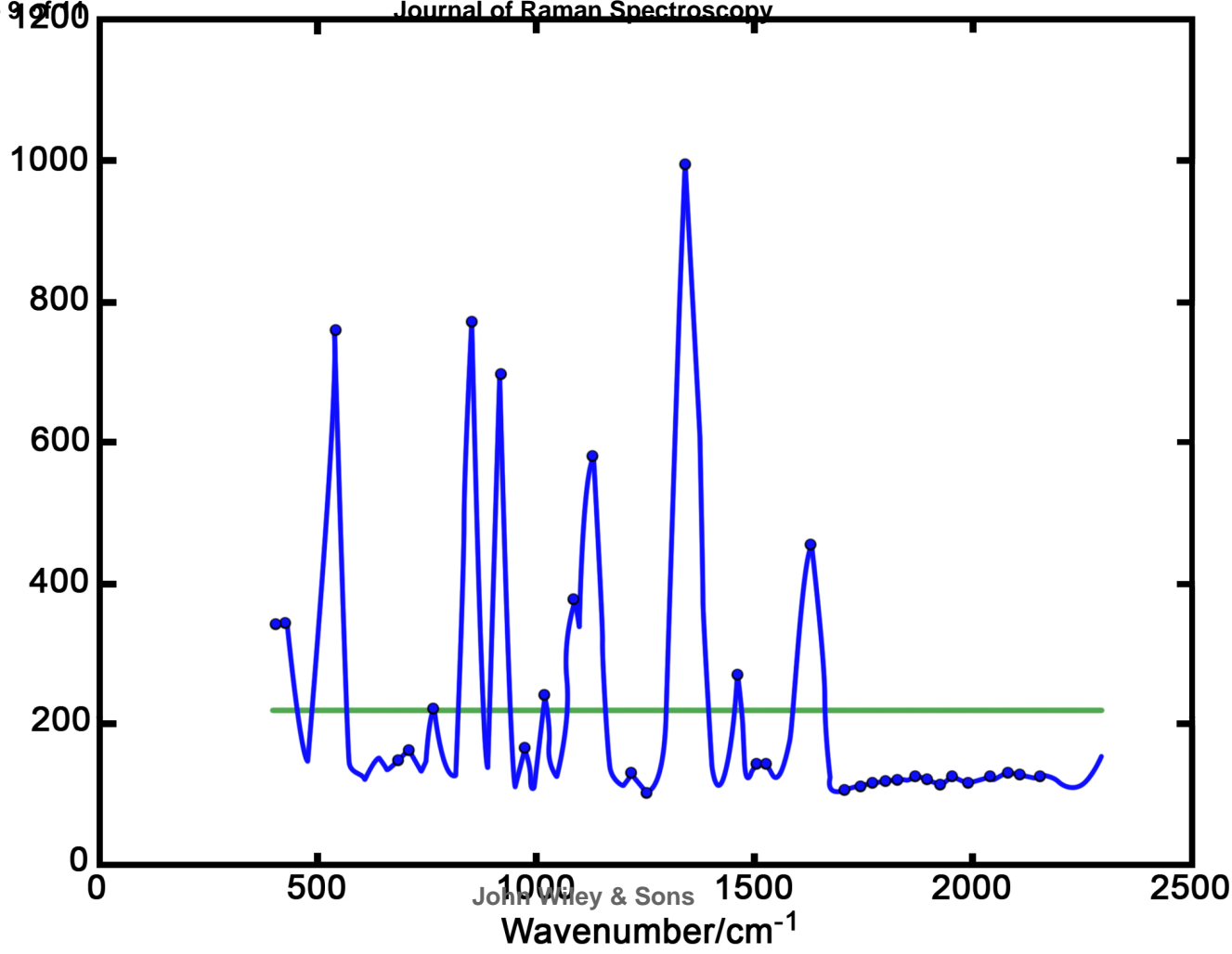
5 182 [14] V. Revin, I. Grunyushkin, N. Gromova, E. Revina, A. S. A. Abdulwahid, I. Solomadin, A. Tychkov and  
6 183 A. Kukina, *Biotechnology & Biotechnological Equipment* **2017**, 31, 128-137.

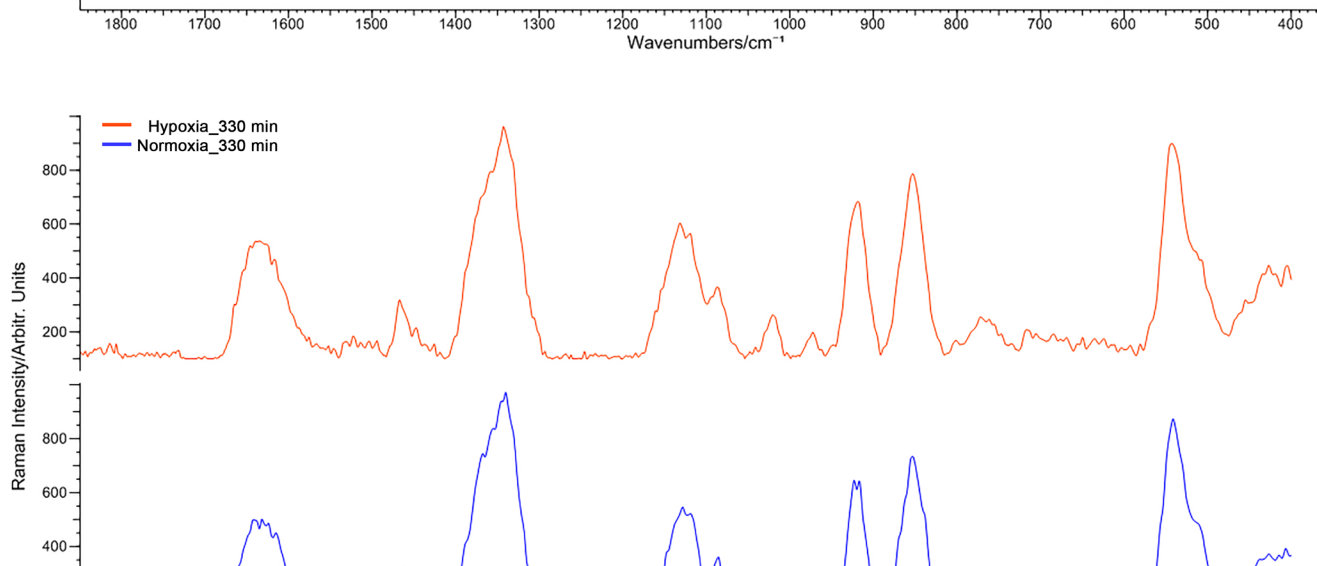
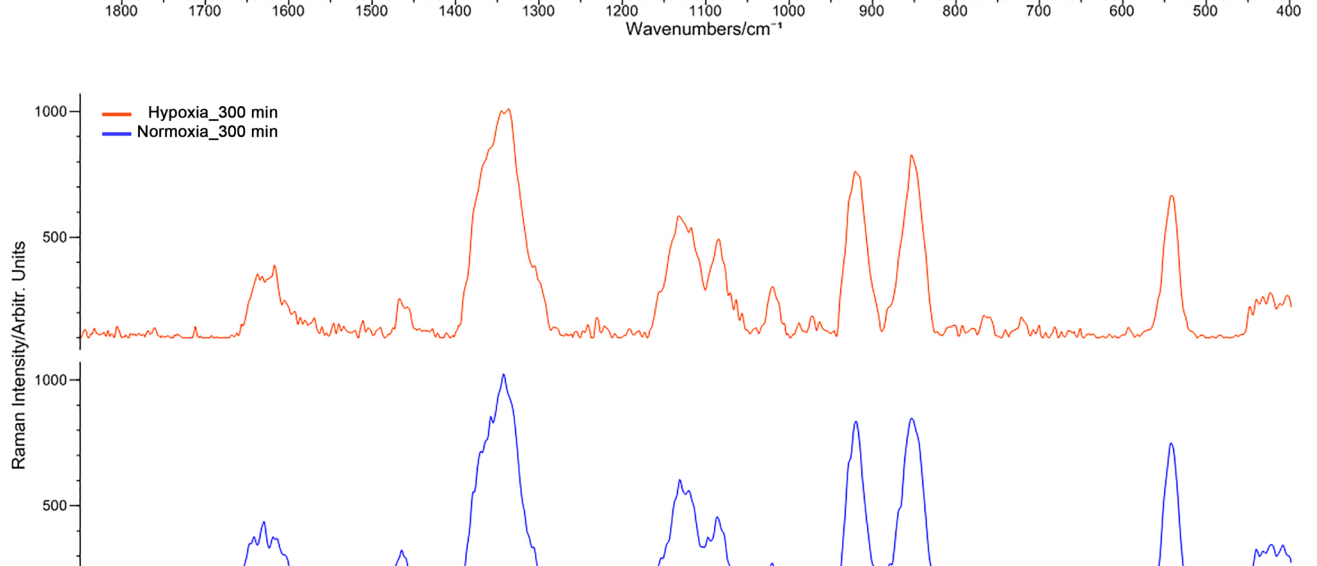
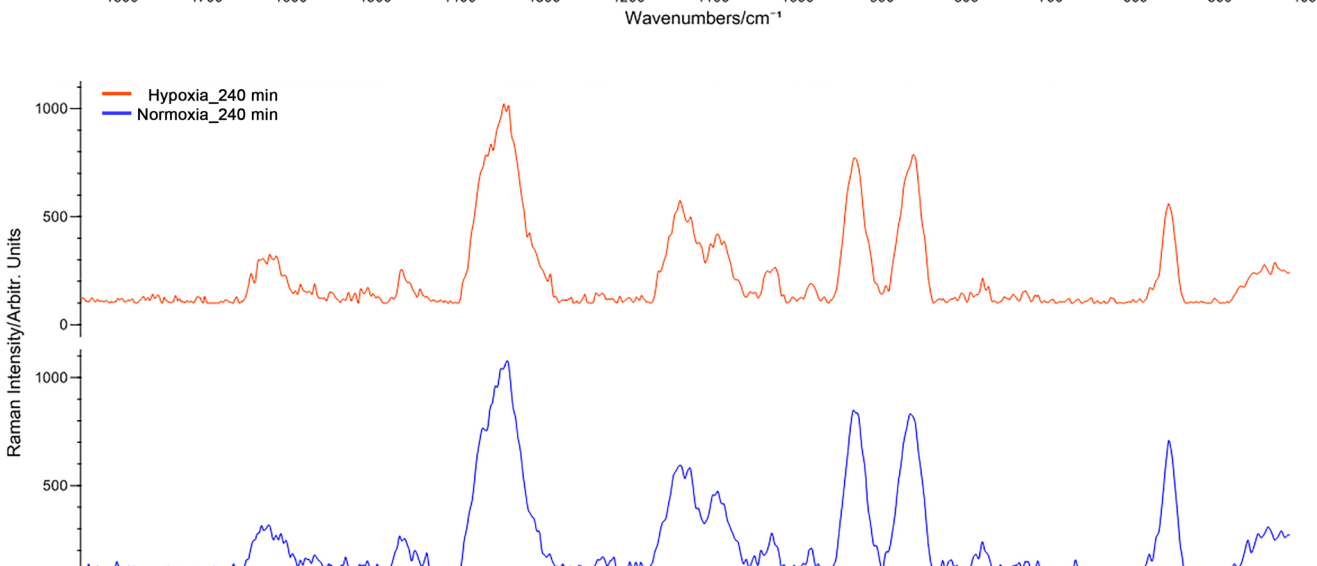
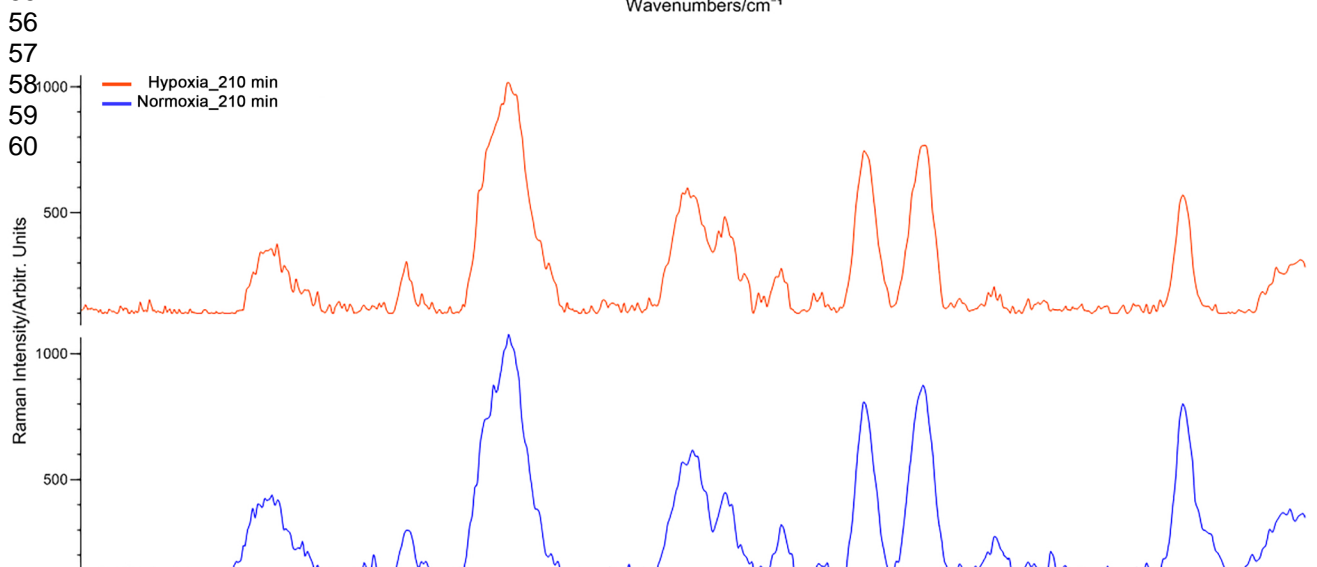
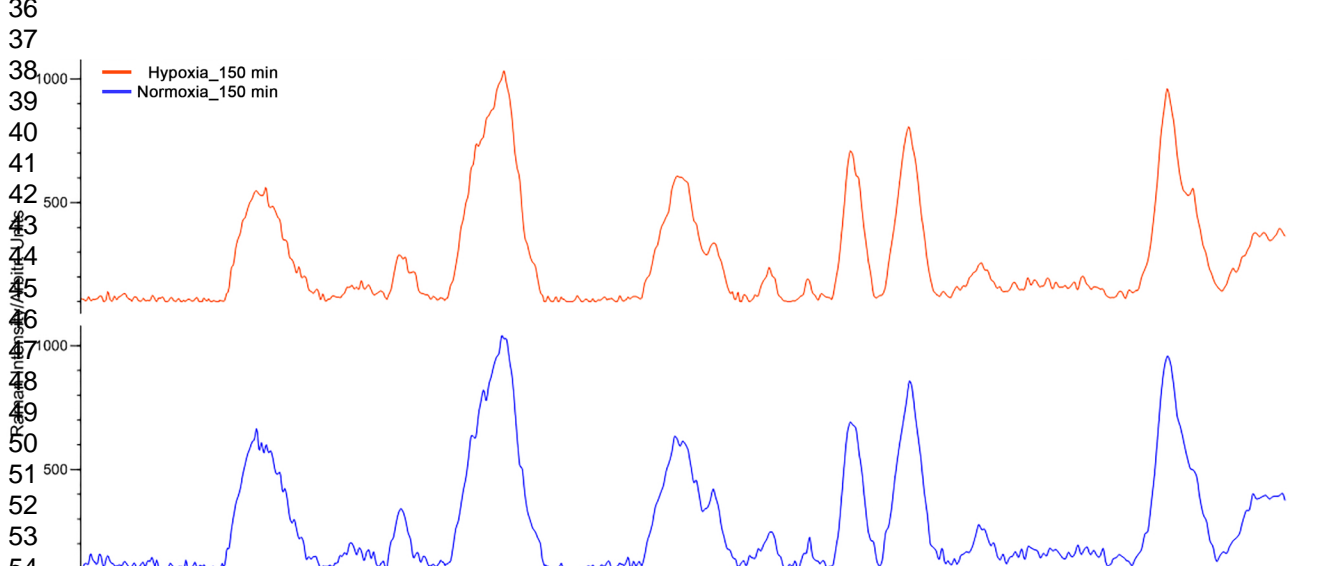
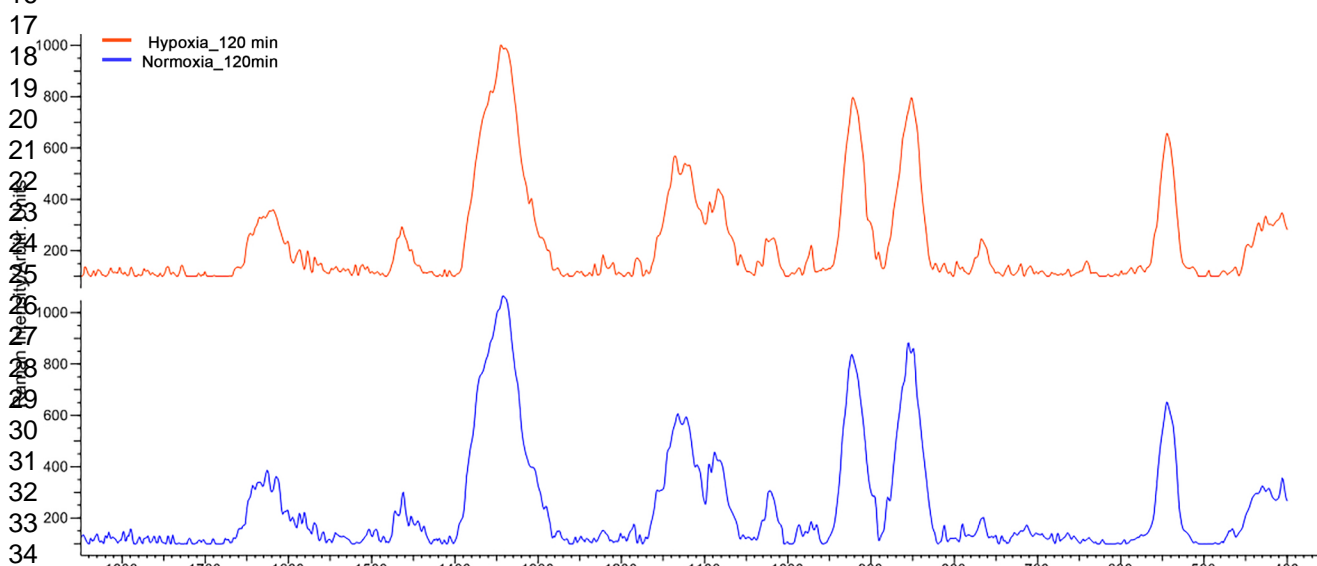
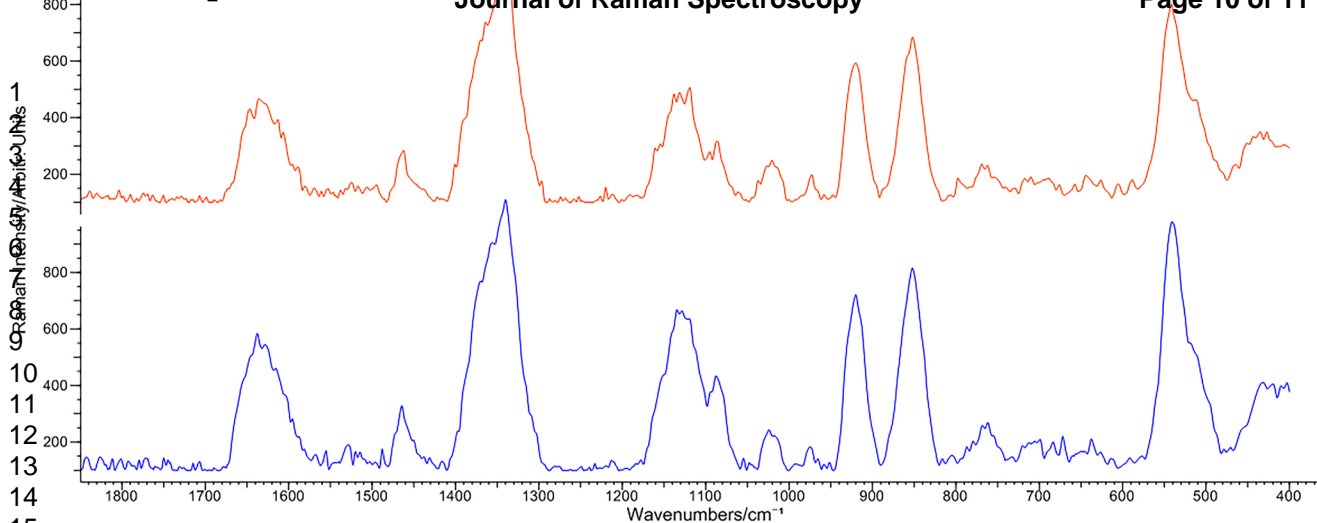
7 184 [15] J. De Gelder, K. De Gussem, P. Vandenabeele and L. Moens, *Journal of Raman Spectroscopy* **2007**,  
8 185 38, 1133-1147.

9  
10  
11 186  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

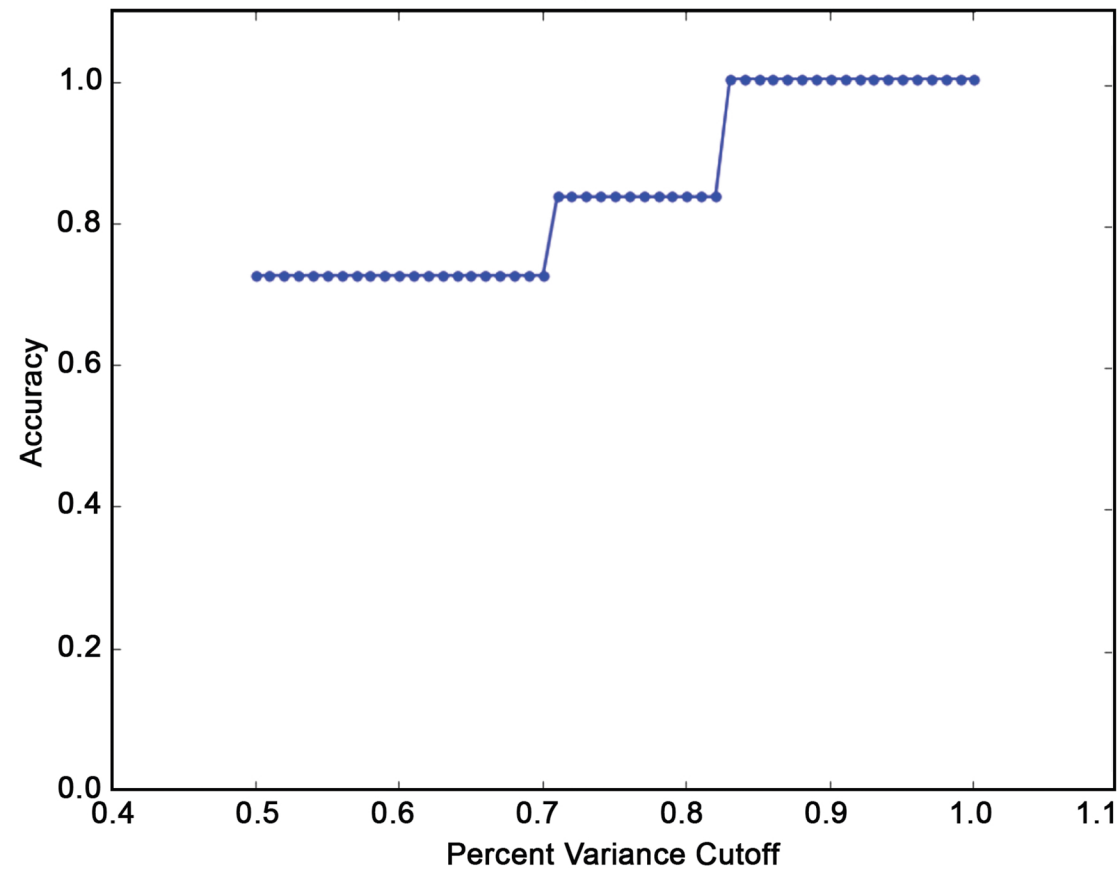
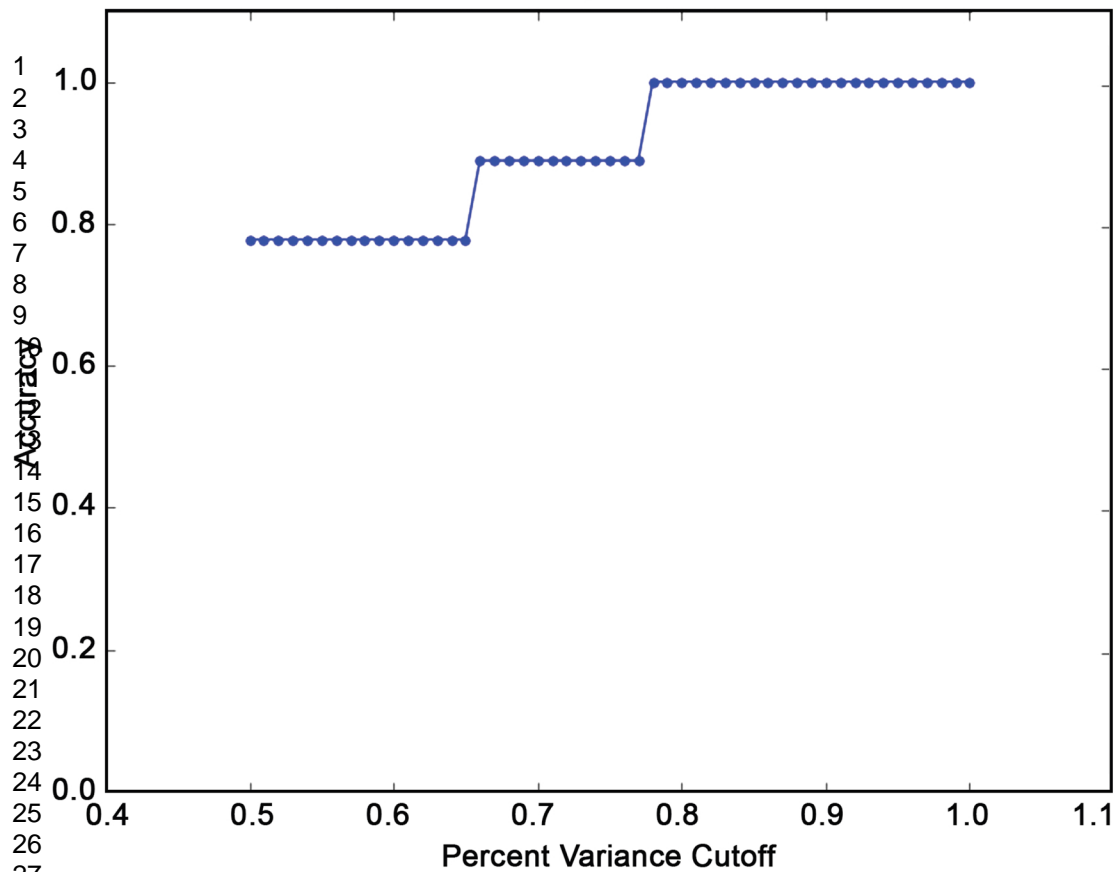
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31





Accuracy at 60 min

Accuracy at 120 min



Accuracy at 150 min

Accuracy at 210 min

