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ARTICLE

Supplementary Materials for *Detection and Time-Tracking Activation of Photosensitisers on Live Single Colorectal Cancer Cells Using Raman Spectroscopy*

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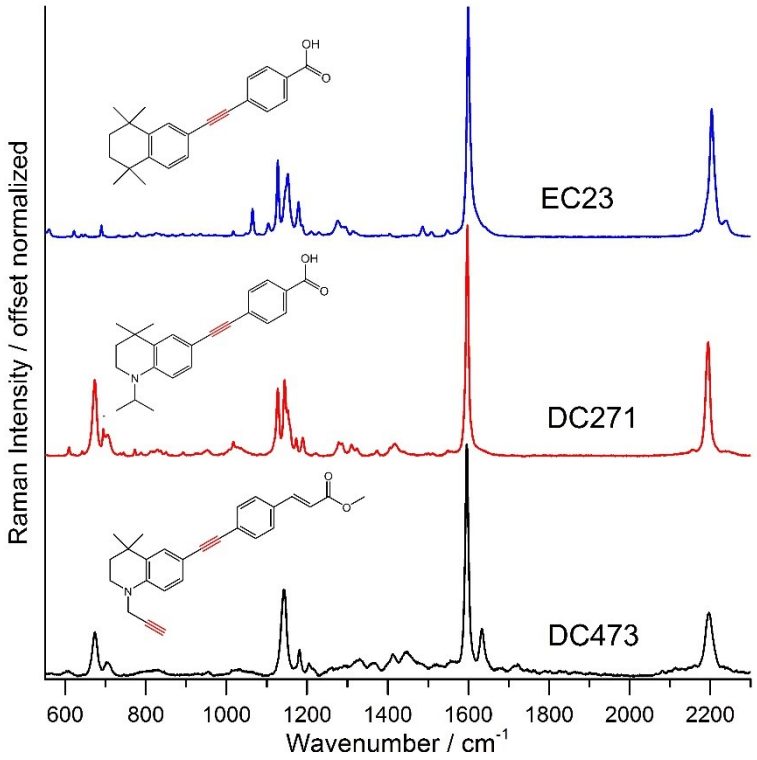

Figure S1: Normalized spectra of crystaline akyne compounds DC273, DC271 and EC23 when exciting with the 785 nm laser line. Compounds were provided by Lightox Ltd. Spectra has been offset for clarity.

Table S1: Bands positions (P), intensities (I) and assignments for DC473, DC271 and EC23 for the crystal spectra acquired with the 785 nm laser shown in Figure S1. Intensity is relative to the maximum intensity of the spectrum, that was the Phe peak at around 1197 cm^{-1} for the three compounds.

DC473		DC271		EC23		Assignment
P / cm^{-1}	I / %	P / cm^{-1}	I / %	P / cm^{-1}	I / %	
305	10	305	10			DMSO
334	12	335	11	337	1	DMSO
		383	8	388	2	DMSO
411	12	424	7	407	2	Tolan/phenyl
453	11	461	10	461	3	Tolan/phenyl
491	11					Ester
		525	4	525	3	Phenyl/carboxylic acid
608	2	610	4	622	3	*Unassigned*
674	18	673	33			DMSO
702	5			690	5	DMSO
		773	3	777	2	Carboxylic acid
829	2	828	3	826	2	*Unassigned*
		892	2	891	1	Carboxylic acid
954	1	952	2	955	1	DMSO
		1018	6	1017	3	Carboxylic acid
1035	2	1085	1	1064	12	DMSO
				1104	6	*Unassigned*
		1127	29	1127	33	Phenyl/carboxylic acid
1141	37	1145	33	1153	27	Phenyl
1181	11	1189	8	1179	15	Phenyl
1204	5	1223	1	1211	2	Phenyl/Tolan
1263	3	1278	6	1277	7	Phenyl/Tolan
1330	7	1310	5	1314	3	Phenyl/Tolan
1367	5	1374	2	1384	1	Phenyl/Tolan
				1404	1	*Unassigned*
1412	9	1418	5			DMSO
1448	10					Ester
				1487	5	*Unassigned*
1521	5	1511	1	1509	2	Phenyl/Tolan
1555	6	1550	2	1548	3	Phenyl/Tolan
1597	100	1598	100	1600	100	Phenyl/Tolan
1634	20					Ester
2197	27	2195	49	2203	56	Alkyne
2234	4	2237	2	2238	7	Alkyne
2874	4	2874	1			Tertiary amine
2916	5	2915	4			DMSO

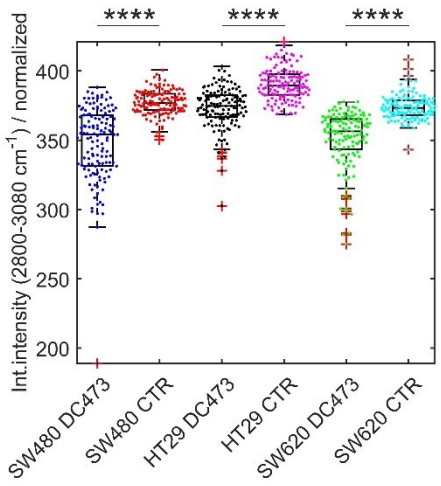

Figure S2: Boxplots and beeswarm plots for the integrated intensity of the single-cell data between 2800 and 3080 cm^{-1} (Data normalized to the Amide I band) following incubation with DC473 or DMSO. Significance level from a normal distribution t-test are indicated (*:p<0.05, **:p<0.01, ***:p<10^{-3}, ****:p<10^{-4})

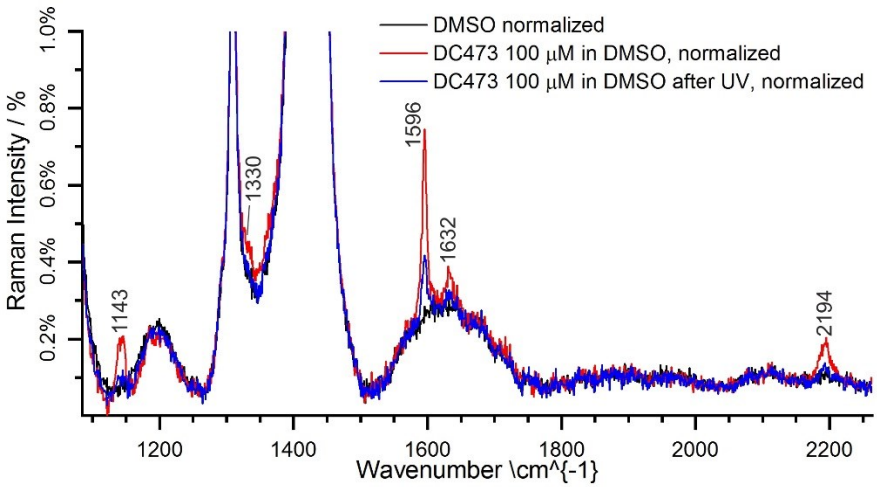

Figure S3: Raman spectra of DC473 (100 µM) in DMSO before and after exposure to UV light (340-360 nm, 5.2 J/cm^2). The DMSO spectrum has been included as a reference, and the main DC473 observed peaks have been labelled. Intensity is expressed as a % of the maximum DMSO band to which the spectrum was normalized.

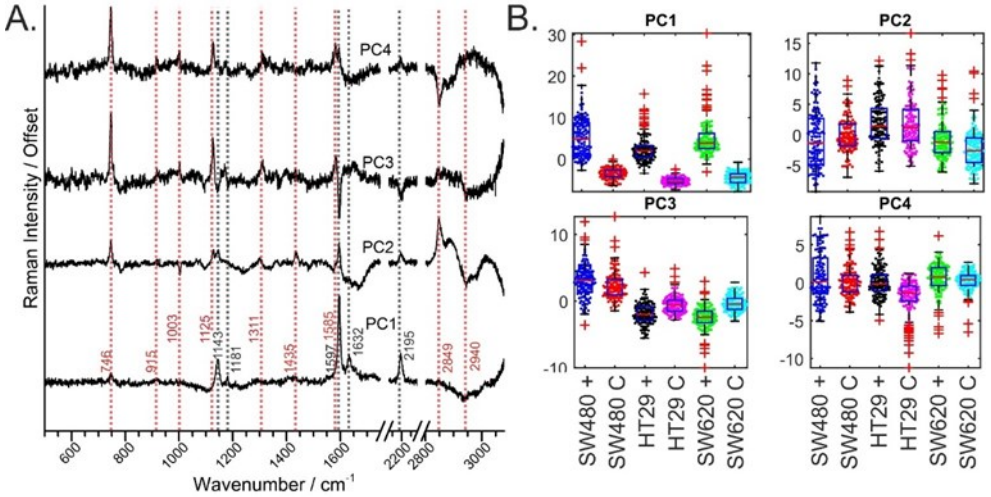

Figure S4: A. First 4 loadings for PCA analysis of live single cell data following incubation with DC473 or DMSO as a control, where the main DC473 peaks (black) and cell peaks (red) have been indicated. B. Boxplots and beeswarm plots for the scores of the first 4 PCs from *A*.

Figure S4A (SI) shows the first 4 PCA loadings explaining 21.5% of the total variability, where the main DC473 (black) and cell (red) peaks have been labelled. Figure S4B (SI) shows the scores for those first 4 PCs. PC1 showed mainly drug related peaks, except for a small contribution of cytochrome C at 746 cm^{-1} and negative contribution in the CH_3 stretching band around 2940 cm^{-1}, confirming that cells with higher concentrations of DC473 displayed higher cytochrome C contributions and lower lipid contribution. PC2 showed significant contributions of cytochrome C peaks, whose scores showed single-cell within population heterogeneity. PC3 showed mainly cytochrome C bands and negative DC473 bands. PC4 showed a drop on the 2849 cm^{-1} CH_2 stretching band with positive cytochrome C bands, showing higher contributions for DC473 incubated cells than control cells, meaning an overall CH stretching drop occurred following incubation with DC473.

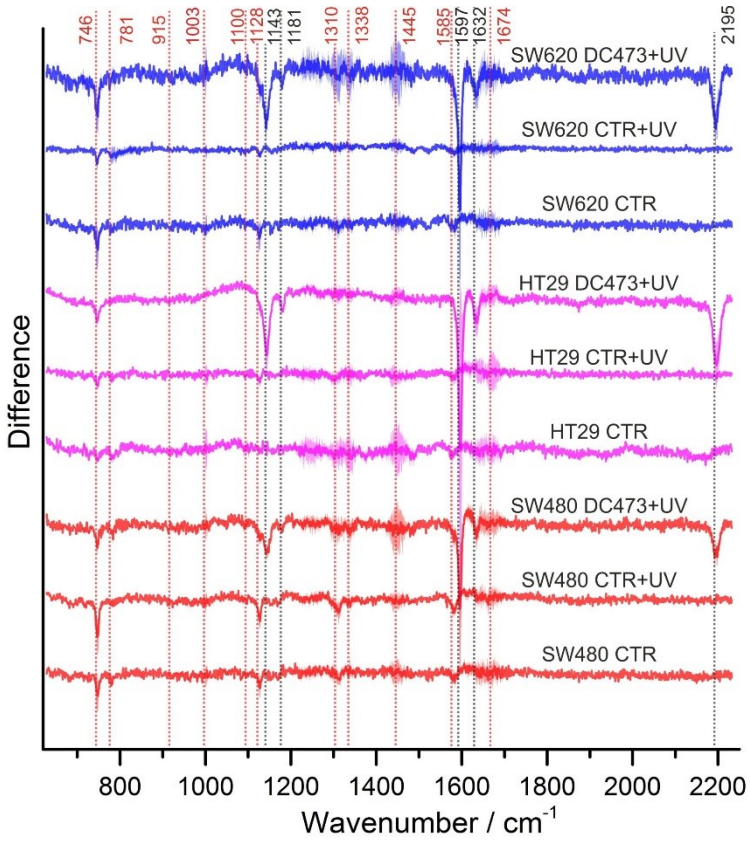

Figure S5: Average difference of the data *last* – *first* spectra for the PDT experiments for each of the samples and cell lines, where CRT=Control, CRT+UV=Control exposed to equivalent dose of UV light, DC473+UV=DC473 sample exposed to UV light. The main bands have been labelled. The area around the curve represents the propagated error.

To explore these changes further, PCA was performed. The variance explained for the first 5 coefficients was 55%. Figure 5A (SI) shows the loadings for the first 5 coefficients, and the main peaks have been labelled. Figure S5 (SI) shows the variation of the average scores for each of the conditions and cell lines with time, where all traces were aligned to 0 for t=0 to better see the trends with time. PC1, similarly to PC5, accounted mostly for substrate variability, showing variability between cells within a cell line and between cell lines, not correlated with the time dependency. PC2 contained the main DC473 contributions, with all the DC473 bands (1443, 1181, 1597, 1632 and 2195 cm^{-1}) present. It showed barely any time changes for the control samples and exponential decay time dependence for the DC473 samples for all cell lines. It also had negative contribution at 746 and 1128 cm^{-1}, indicating a possible negative correlation with cytochrome C, likely due to the presence of multiple observations post PDT where the DC473 bands were weakly present and the cytochrome C bands dropped. The biggest drop in PC2 was on HT29 cells, followed by SW620 cells and then SW480 cells.

PC3 contained positive cytochrome C bands (746, 1128 and 1585 cm^{-1}) and negative contributions in the region around 780-840 cm^{-1}, at the Phe band at 1003 cm^{-1} and a broad positive contribution at around 1455 (lipids) and 1656 (Amide I) cm^{-1}. This coefficient could be indicating that cytochrome C bands correlated with lipids and protein. The scores of PC3 showed a slight and constant negative slope for the control samples, with a more drastic exponential-like reduction for the DC473+UV sample immediately after PDT. The HT29 and SW620 PDT samples showed significant drops in PC3 scores, with smaller changes for the SW480 PDT sample, that showed a similar trend to the SW480 CTR+UV sample.

PC4 had a similar loading and trend to PC3, but with a positive band around 780-840 cm^{-1}, a negative band around 1455 cm^{-1} and slightly negative contribution around 1656 cm^{-1}. Additionally, it showed a series of repetitive bands at 1314, 1338, 1362 and 1390 cm^{-1}. Again, HT29 and SW620 cells showed a stronger reduction in this coefficient after PDT, with lower responses and overlap of SW480 DC473+UV and CTR+UV samples.

PCA was unable to identify any clear Raman marker for the cell blebbing morphology changes observed after PDT, and only showed a strong reduction on PC3 and 4 on all treated cells, in line with the amount of DC473 that the cells accumulated according to previous experiments.

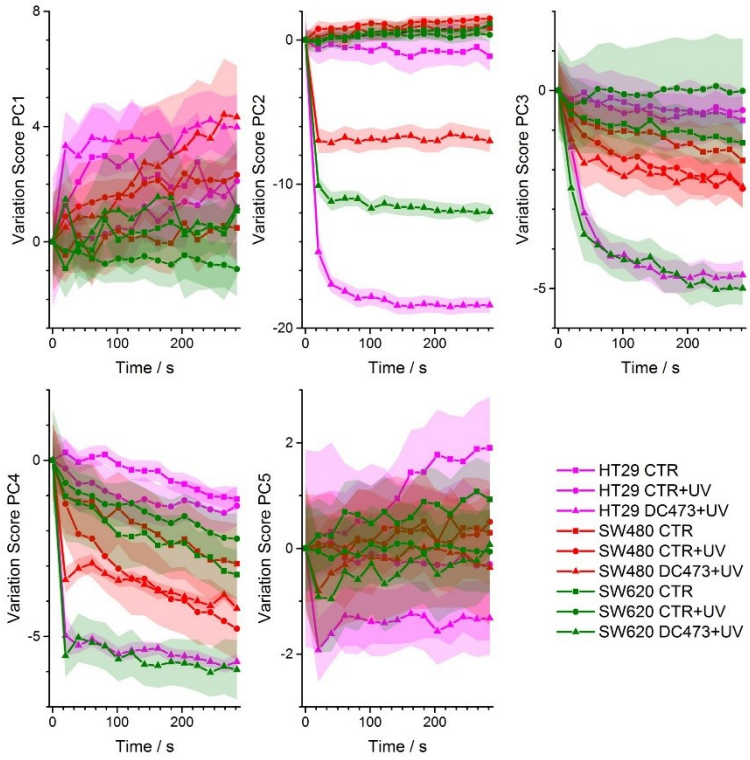

Figure S6: PCA average score-time traces for the first 5 PCs for the PDT data, whose loadings are shown in Figure 5A. All curves have been translated so the first point of each curve is at zero to better see the time trends. The area around the curve represents the standard error.