Supporting Information

Towards Single Organelle Lipidomics in Live Cells

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Abstract

Detailed studies of lipids in biological systems including their role in cellular structure, metabolism and disease development, comprise an increasingly prominent discipline called lipidomics. However, the conventional lipidomics tools, such as mass spectrometry, cannot investigate lipidomes until they are extracted, and thus cannot be used neither for probing the lipids distribution, nor for studying in live cells. Furthermore, conventional techniques rely on the lipid extraction from relatively large samples, which averages the data across the cellular populations and masks essential cell-to-cell variations. Further advancement of the discipline of lipidomics critically depends on the capability of high-resolution lipid profiling in live cells and, potentially, in single organelles. Here we report micro-Raman assay designed for single organelle lipidomics. We demonstrate how Raman microscopy can be used to measure the local intracellular biochemical composition and lipidome hallmarks – lipids concentration and unsaturation level, cis/trans isomers ratio, as well sphingolipids and cholesterol levels in live cells, with a submicron resolution, which is sufficient for profiling of subcellular structures. These lipidome data were generated by a newly developed Biomolecular Component Analysis software, which provides a shared platform for data analysis among different research groups. We outline a robust, reliable and user-friendly protocol for quantitative analysis of lipid profiles in subcellular structures. This method expands the capabilities of Raman-based lipidomics towards the analysis of single organelles within either live or fixed cells, thus allowing an unprecedented measure of organellar lipid heterogeneity, and opening new quantitative ways to study the phenotypic variability in normal and diseased cells.

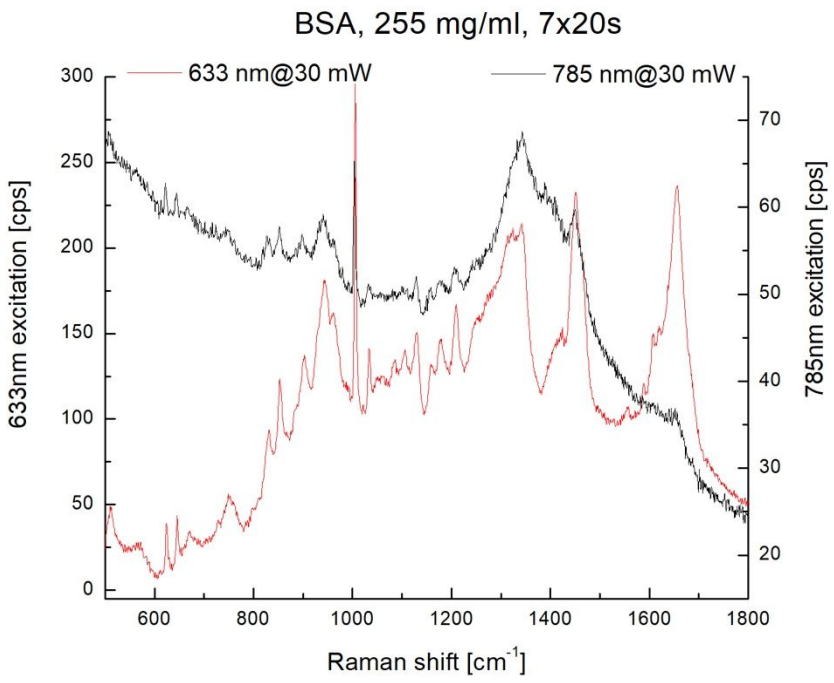

Figure S1. Raman spectra of the same sample (Bovine serum albumin water solution) excited by 633 nm and 785 nm laser modules from ThermoFisher Scientific. Spectrum generated by 785 nm laser demonstrates significantly larger background in the area 1200-1500 cm^{-1}

Comment to Fig S1: To evaluate the advantages and drawbacks of Raman excitation at 633 and 785 nm, we measured Raman signal from the same sample (255 mg/ml Bovine serum albumin water solution). The result is shown in Fig.1S. We found that the ratio of signal to background for measurements at 785 nm was surprisingly higher than that for 633 nm (Fig 1a), since we expected less cellular autofluorescence and reduced scattering at the longer wavelength of 785 nm. An increased background level for the 785 nm excitation laser might be resulted from autofluorescence and Raman scattering from impurities in the glass substrate, as it has been seen by others. Using the 785 nm laser as excitation source decreases noticeably the accuracy of the background subtraction, which, in turn, increases BCA resulting error.

a) b)

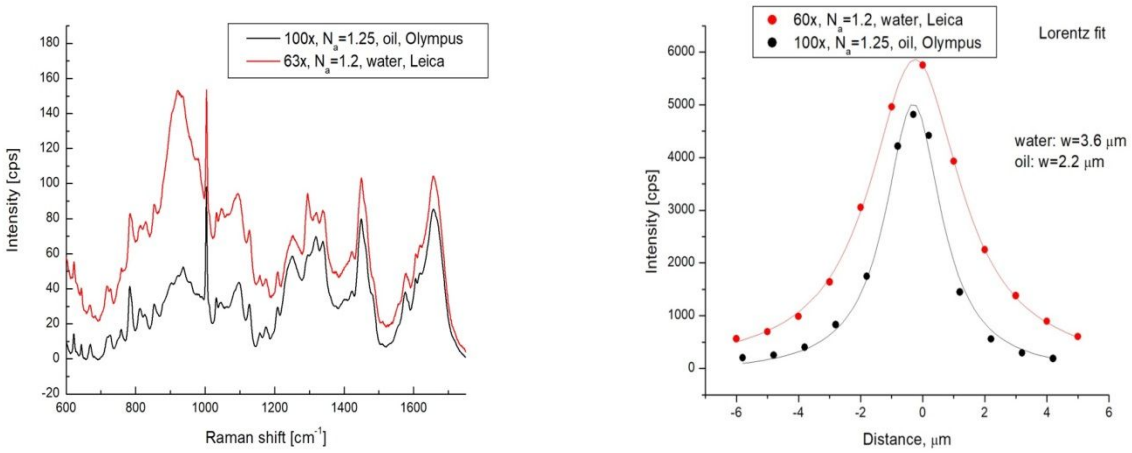

Figure S2. Raw Raman spectra of the same nucleolus (the same cell) measured using 100^x (N_a=1.25, oil immersion, black curve) Olympus and 63^x (N_a=1.20, water immersion, red curve) Leica objective lenses (a). Parameter of confocality for oil immersion 100x objective lens is about 2.2 μm, while that for water immersion 63x objective lens is about 3.6 μm (b). Therefore, measurement of the same sample under the same measurement conditions resulted in larger autofluorescence and glass background signal for water immersion objective lens. Confocal parameters for two objective lenses were measured by scanning over thin polystyrene film (~200 nm) along Z-axis; confocal pinhole: 50 μm

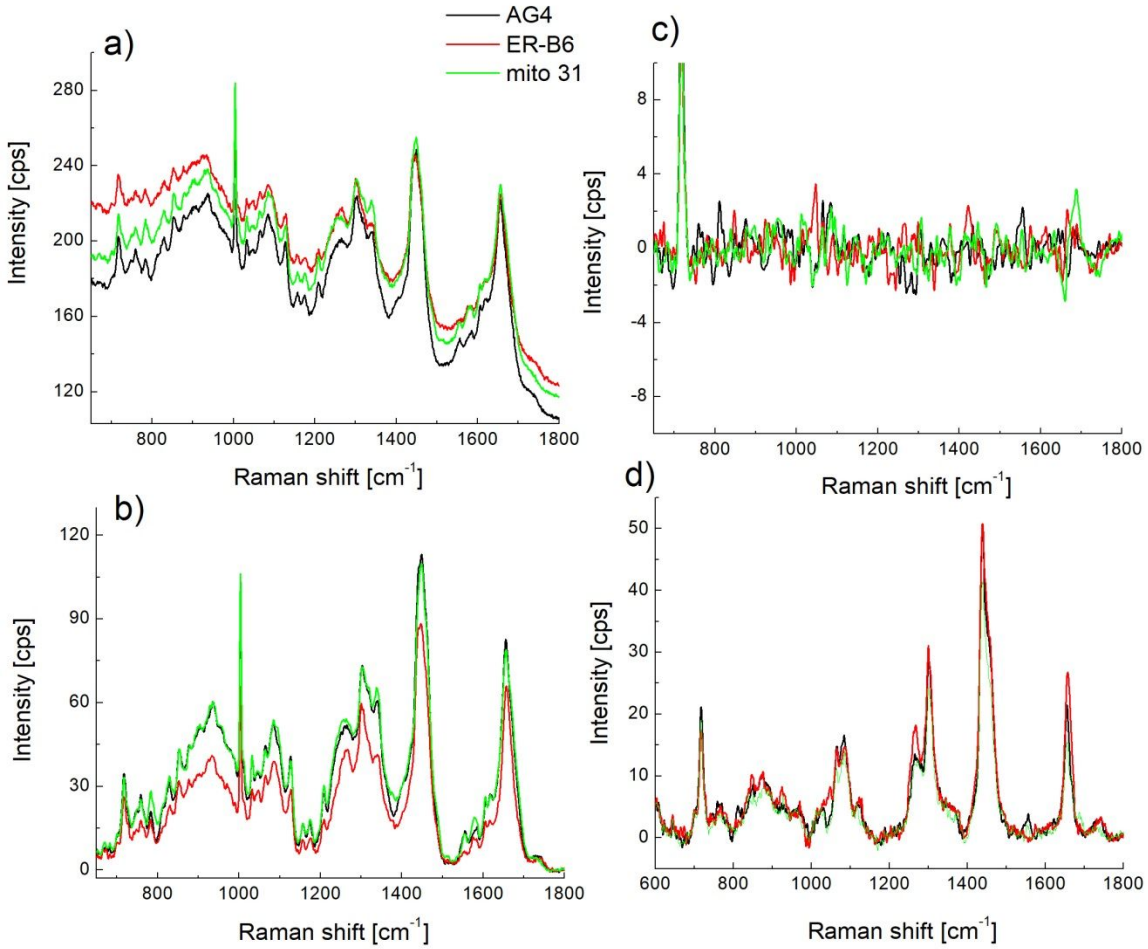

Figure S3. Raman spectra of cellular organelles. Representative raw (a) and pre-processed (b) Raman spectra of cytoplasmic organelles – Apparatus Golgi (black), Endoplasmic Reticulum (red) and Mitochondrion (green) in live HeLa cell. The effects of software pre-processing on spectra, which represents residual spectra of corresponding organelle obtained using the BCAbox is shown in panel (c). Organellar lipids Raman spectrum, extracted from spectra illustrated in (b) by BCAbox are shown in panel (d).

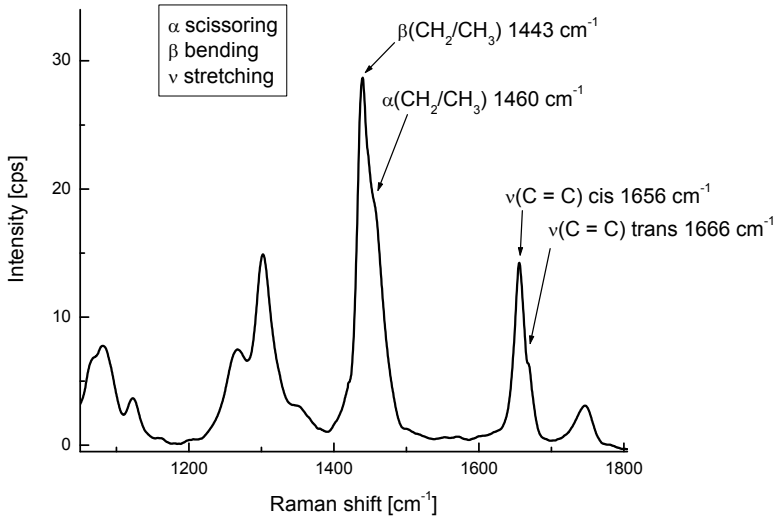

Figure S4. Raman spectrum of HeLa AG cellular lipids in the range between 1100 and 1800 cm^{-1}. Arrows show assigned vibration modes.

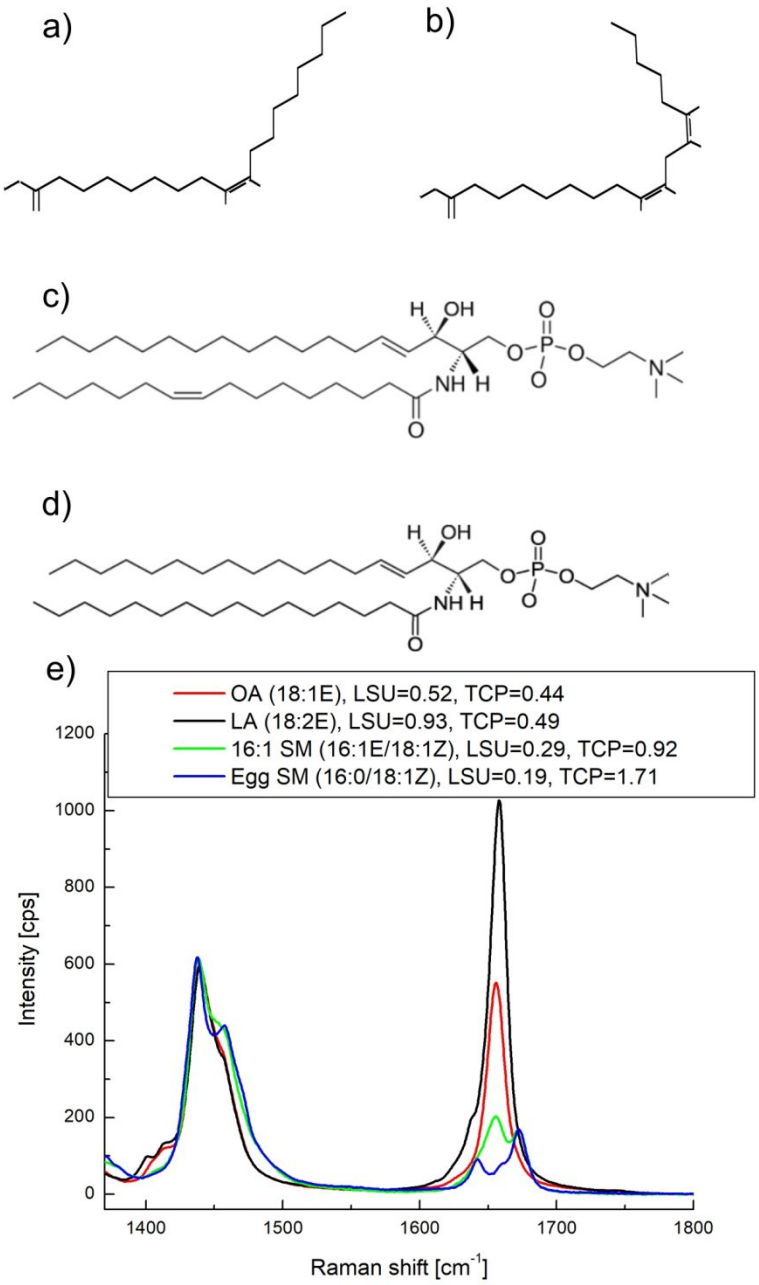

Figure S5. Structures of (a) monounsaturated oleic (18:1E), (b) polyunsaturated linoleic (18:2E) acids with the same number of carbon atoms, (c) N-palmitoleoyl-D-erythro-sphingosylphosphorylcholine (16:1E/18:1Z) with one cis and one trans C=C bonds, and (d) chicken egg sphingomyelin (16:0/18:1Z) with one trans C=C bond. Corresponding Raman spectra in the range between 1370 and 1800 cm^{-1} are shown in (c) panel. Abbreviations: OA – oleic acid, LA – linoleic acid, Egg SM – chicken egg sphingomyelin, 16:1 SM - N-palmitoleoyl-D-erythro-sphingosylphosphorylcholine, LSU – lipids unsaturation parameter, TCP – lipids trans/cis parameter. The numbers in parenthesis show a number of carbon bonds (first) and a number of double bonds (second); letter after the second number denotes cis- (E) or trans- (Z) isomer of double bond.

Materials: oleic and linoleic acids from Sigma (St. Louis, MO); N-palmitoleoyl-D-erythro-sphingosylphosphorylcholine and chicken egg sphingomyelin from Avanti Polar Lipids (Alabaster, AL).

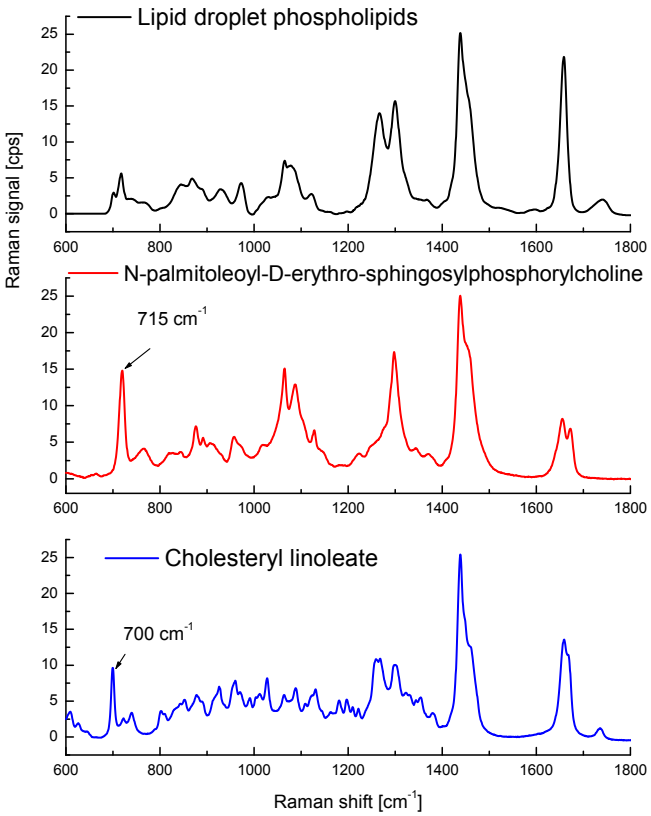

Figure S6. Raman spectra of typical lipid droplet in HeLa cell (upper panel), N-palmitoleoyl-D-erythro-sphingosylphosphorylcholine (middle panel) and Cholesteryl linoleate (lower panel). Characteristic peaks used for identification of sphingolipids and cholesterols are shown by arrows.

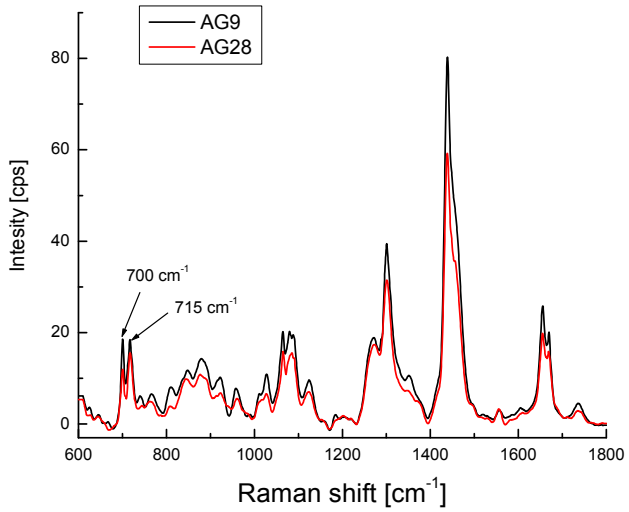

Figure S7. Raman spectra of AG lipids in HeLa cells, extracted by BCAbox. Characteristic peaks of sphingolipids and cholesterols are clearly identified (shown by arrows).

Table S1. One-way ANOVA ($p<0.05$) for sets of LSU, TCP and PC/SM of lipids in AG, mitochodrion, ER and LD for HeLa cells. Result **Y/N** means if mean values are significantly different.

		AG	ER	ER	mito	AG	LD
LSU	mean	0.290	0.350	0.350	0.370	0.290	0.280
	variance	9.87E-4	0.002	0.002	0.003	9.87E-4	5.18E-4
	F	75.59		2.27		1.18	
	p	4.81E-14		0.137		0.28	
	result	**Y**		**N**		**N**	
TCP	mean	0.560	0.580	0.580	0.640	0.560	0.44
	variance	0.005	0.014	0.014	0.003	0.006	0.002
	F	0.849		3.84074		49.10601	
	p	0.35892		0.0543		3.6659E-9	
	result	**N**		**N**		**Y**	
PC/SM	mean	0.630	0.690	0.690	0.699	0.630	0.12
	variance	0.016	0.037	0.037	0.051	0.016	0.002
	F	3.455		0.01766		341.26471	
	p	0.06583		0.89468		0	
	result	**N**		**N**		**Y**	

Table S2. One-way ANOVA ($p<0.05$) for sets of LSU, TCP and PC/SM of lipids in AG and LD for HeLa, U251 and BMEC cell lines. Result **Y/N** means if mean values are significantly different.

		HeLa	U251	HeLa	BMEC	U251	BMEC
AG LSU	mean	0.287	0.502	0.287	0.462	0.502	0.462
	variance	9.86E-4	0.008	9.86E-4	0.003	0.008	0.004
	F	277.46		288.62		3.87029	
	p	0		0		0.05342	
	result	**Y**		**Y**		**N**	
AG TCP	mean	0.560	0.541	0.560	0.640	0.541	0.640
	variance	0.006	0.004	0.006	0.011	0.004	0.011
	F	1.804		14.69592		24.96267	
	p	0.1823		2.60852E-4		4.67017E-6	
	result	**N**		**Y**		**Y**	
AG PC/SM	mean	0.632	0.695	0.632	0.727	0.695	0.727
	variance	0.016	0.040	0.016	0.030	0.040	0.030
	F	3.59971		7.27693		0.42885	
	p	0.06079		0.00862		0.51487	
	result	**N**		**Y**		**N**	
LD LSU	mean	0.279	0.433	0.279	0.369	0.433	0.369
	variance	5.177E-4	0.008	5.177E-4	0.002	0.008	0.002
	F	34.46502		51.90579		2.11368	
	p	4.52293E-7		7.66965E-8		0.15573	
	result	**Y**		**Y**		**N**	
LD TCP	mean	0.444	0.576	0.444	0.5238	0.576	0.5238
	variance	0.002	0.005	0.002	0.037	0.005	0.037
	F	62.37682		3.54799		1.42211	
	p	4.18414E-10		0.07004		0.24182	
	result	**Y**		**N**		**N**	
LD PC/SM	mean	0.119	0.337	0.119	0.059	0.337	0.059
	variance	0.002	0.014	0.002	0.0011	0.014	0.014
	F	68.0934		12.28978		43.71555	
	p	1.26124E-10		0.00155		1.86967E-7	
	result	**Y**		**Y**		**Y**	

Table S3. One-way ANOVA ($p<0.05$) for sets of LSU, TCP and PC/SM of lipids in ER and LD of U251R132C before and after drug application. Result **Y/N** means if mean values are significantly different.

		U251R132C control	U251R132C drug
ER Lipids	mean	0.786	1.670
	variance	0.027	0.450
	F	30.88463	
	p	4.34381E-6	
	result	**Y**	
ER LSU	mean	0.586	0.371
	variance	0.029	0.006
	F	19.45704,	
	p	1.15213E-4	
	result	**Y**	
LD TCP	mean	0.583	0.508
	variance	0.004	0.001
	F	5.95846	
	p	p = 0.02149	
	result	**Y**	
LD PC/SM	mean	0.334	0.014
	variance	5.177E-4	0.008
	F	6.14259	
	p	0.01974	
	result	**Y**	