Supporting Information (SI)

In vivo Lipidomics Using Single-Cell Raman Spectroscopy

Huawen Wu[a], Joanne V. Volponi[a], Ann E. Oliver[b], Atul N. Parikh[b], Blake A. Simmons[a] and Seema Singh[a,1]

[a] Biomass Science and Conversion Technology Department, Sandia National Laboratories, Livermore, CA 94551, USA

[b] Department of Applied Science, University of California, Davis, CA 95616, USA

[1] To whom correspondence may be addressed at: MS 9292, PO Box 969, Livermore, CA 94551. Email: seesing@sandia.gov

Fig. S1.

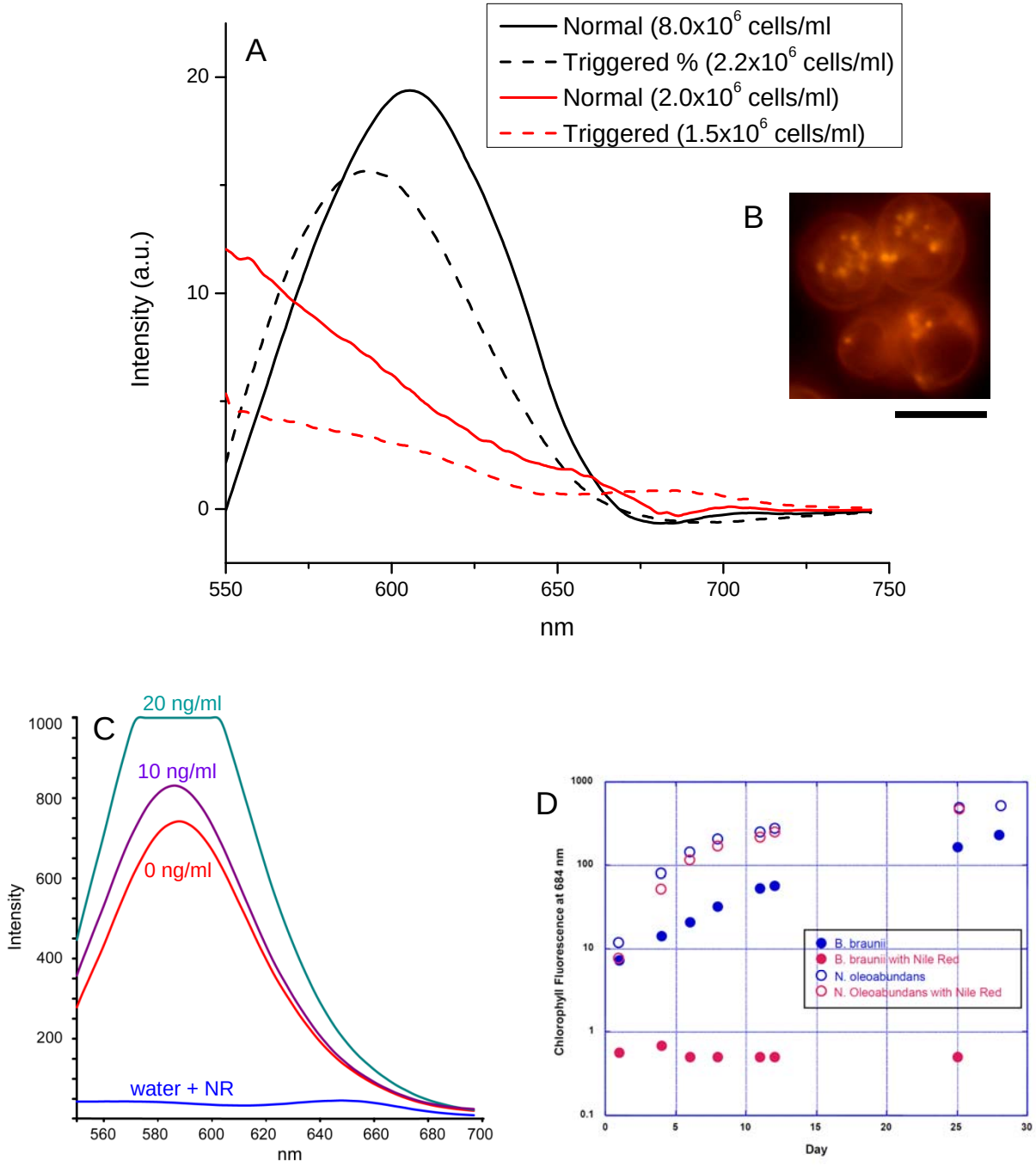


Fig. S1. Nile Red staining technique is demonstrated here for the applications on *N. oleoabundans* cells. However, it is also found misleading and not applicable for other species. (*A*) Blue-shifted Nile Red emission peak from Nile Red labeled *N. oleoabundans* cell suspension after lipid "trigger" of nitrate starvation indicates increased amount of TAGs in the cells.

However, due to the limited sensitivity, the results are only stable when *N. oleoabundans* cell density is sufficiently high (> ~ 2×10^6 cells/ml). (*B*) Nile Red labeled normal *N. oleoabundans* cells imaged by a fluorescent microscope, scale bar = 5 μm. (*C*) Presence of carotenoids misleads Nile Red measurement. Same amount of TAGs (olive oil at 50 μg/ml) mixed with different labeled amounts of β-carotene yields dramatically different Nile Red emission. (*D*) Nile Red staining is species-specific as demonstrated in the growth curves (marked by chlorophyll fluorescence at 684 nm) of *N. oleoabundans* and *B. braunii* cultured with and without the presence of Nile Red. Nile Red is applicable to *N. oleoabundans* but is toxic to *B. braunii*.

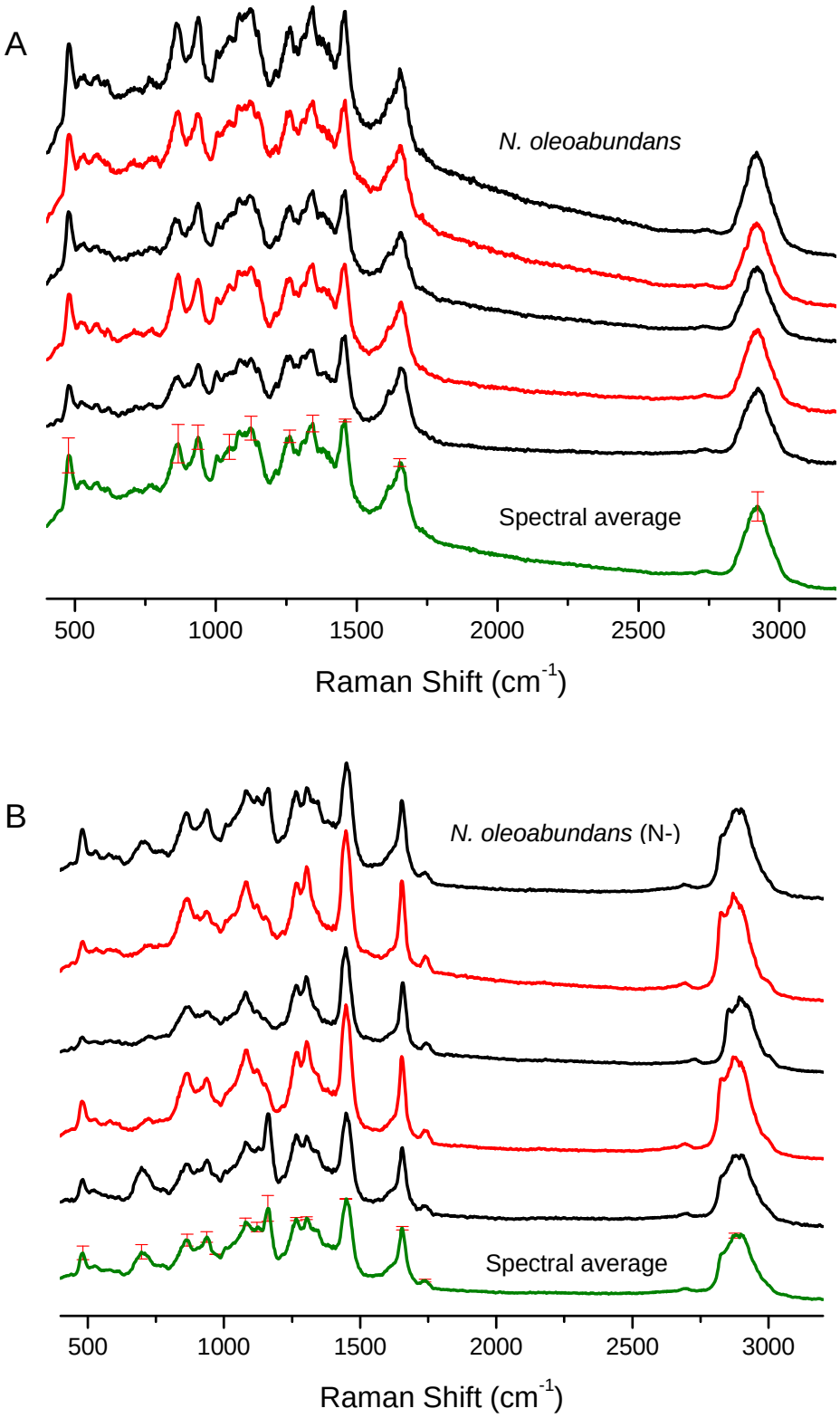
Method

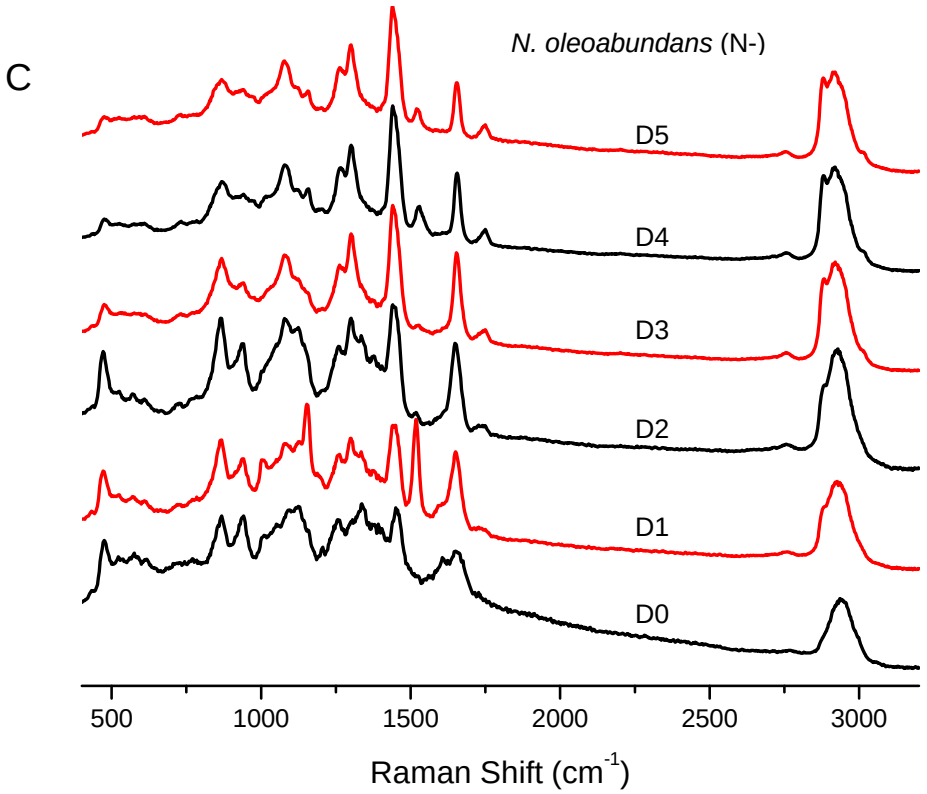
To stain microalgal cells, 2 ml of cell suspension was transferred to a plastic cuvette, with 8 μl of 250 μg/ml Nile Red added. Using an excitation wavelength of 525 nm, fluorescent emission from 550 to 750 nm was monitored. A broad emission peak will develop at approximately 580 nm for TAGs, or at 620 nm for phospholipids. Since algal strains vary in the length of time necessary to reach maximal staining with Nile Red, the scans were run several times until no further change was seen. In order to obtain data relevant only to the staining of algae with Nile Red, the fluorescent emission scans were corrected by subtracting scans of growth medium stained by Nile Red and the samples without Nile Red labeling.

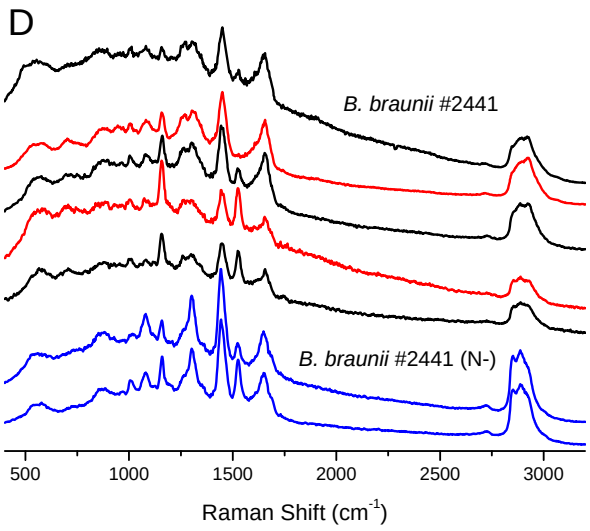
To stain β-carotene tainted oil samples, β-carotene (Sigma-Aldrich C9750) dissolved in acetone (approximately 1 μg/ml) is mixed with olive oil to labeled concentrations and sonicated for 2 minutes before Nile Red labeling and measurements.

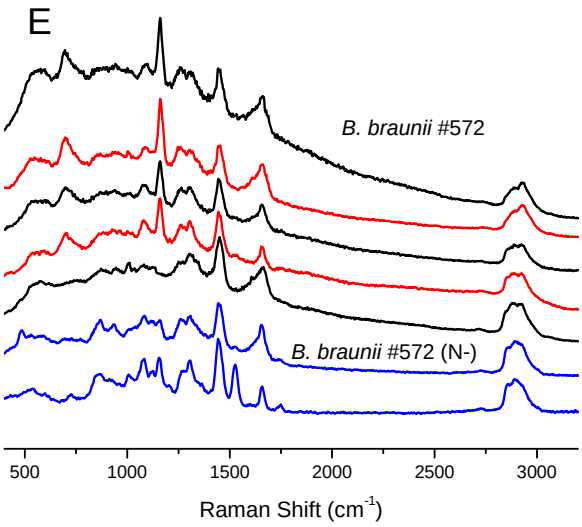
To growing microalgae in the presence of Nile Red, 120 ml of algal culture plus 480 μl of 250 μg/ml Nile Red (#N-1142, Invitrogen, Carlsbad, CA) dissolved in acetone was placed in 250-ml sterile glass flasks loosely capped with foil. Control cultures (no Nile Red) were maintained under identical conditions. In addition, same growth media with Nile Red were maintained under identical conditions for use in assay background subtractions. Algal growth was monitored by measuring chlorophyll fluorescence. Aliquots (2 ml) of the cultures were transferred to cuvettes and the fluorescence emission was measured with a Perkin Elmer Fluorescence Spectrometer Model LS 55 using excitation at 430 nm, and an emission window from 600 to 800 nm. Chlorophyll produces an emission peak at approximately 684 nm. Fluorescence emission scans were corrected by subtracting emission scans taken on cuvettes containing growth medium only.

Fig. S2.


A
N. oleoabundans
Spectral average
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)
B
N. oleoabundans (N-)
Spectral average
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)


C
N. oleoabundans (N-)
D5
D4
D3
D2
D1
D0
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)


D
B. braunii #2441
B. braunii #2441 (N-)
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)


E
B. braunii #572
B. braunii #572 (N-)
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)

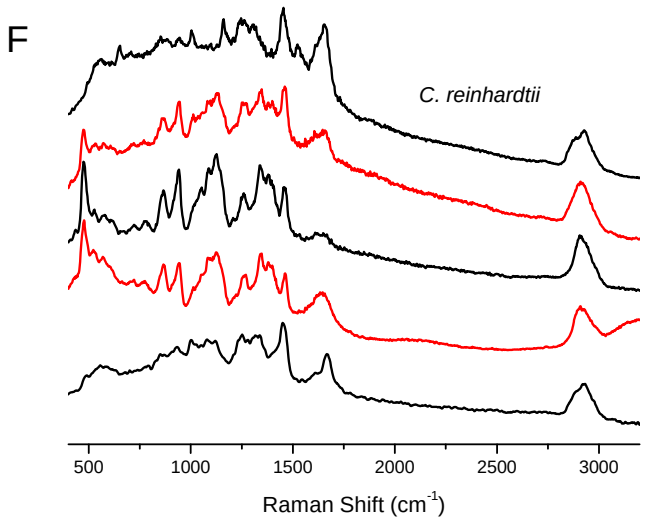


Fig. S2. LTRS spectra of several individual microalgal cells in a small population indicating bulk characteristics as well as cell-cell variations. (*A*) *N. oleoabundans* cells in normal condition (*black and red*) and their spectral average with standard deviation (*green*). (*B*) Lipid triggered *N. oleoabundans* cells in nitrate starvation condition (*black and red*) and their spectral average with standard deviation (*green*). (*C*) A time course of the spectral average (of five cells each) of *N. oleoabundans* cells cultured in nitrate starvation condition from beginning (D0) to the 5th day (D5) of the growth. (*D*) *B. braunii* #2441 cells in normal condition (*black and red*) and in nitrate starvation condition (*blue*). (*E*) *B. braunii* #572 cells in normal condition (*black and red*) and in nitrate starvation condition (*blue*). (*F*) *C. reinhardtii* cells in normal condition (*black and red*).

Table S1.

Species	I_{1650}/I_{1440}	Avg. lipid chain length (± s.d.)	Degree of unsaturation (avg. number of C=C) (± s.d.)	T_m (°C) (± s.d.)
N. oleoabundans (UTEX #1185)	0.75	14.3 ± 0.9	0.97 ± 0.04	2.6 ± 1.9
B. braunii (UTEX #572)	0.39	14.6 ±1.5	0.44 ± 0.03	9.5 ± 2.9
B. braunii (UTEX#2441)	0.44	14.4 ±1.3	0.51 ± 0.03	8.1 ± 2.7

Table S1. Lipid characteristics including average lipid chain length, degree of unsaturation (average number of C=C bonds per lipid molecule) and melting temperatures (T_m) are derived from the spectroscopic marker I_{1650}/I_{1440}, for individual microalgae cells of three species cultured in nitrate-depleted media.

Note S1, Fitting Parameters in Quantitative Raman Spectral Analysis

Linear regression fitting in Fig. 4A:

$I_{1650}/I_{1440} = A + B \times N_{CH2}/N_{C=C}$ (eq. S1)

Parameter	Value	Error
A	0.15516	0.03279
B	6.34291	0.19027

R	SD	N	P
0.99598	0.08999	11	<0.0001

Linear regression fitting in Fig. 4B:

$I_{1650}/I_{1440} = A + B \times N_{C=C}$ (eq. S2)

Parameter	Value	Error
A	0.09809	0.03241
B	0.671	0.0193

R	SD	N	P
0.9963	0.08633	11	<0.0001

Boltzmann fitting in Fig. 4C:

$$I_{1650} / I_{1440} = \frac{A_1 - A_2}{1 + e^{(T_m - x_0)/dx}} + A_2$$ (eq. S3)

Parameter	Value	Error
A_1	2.86921	0.15414
A_2	0.14833	0.06549
x_0	-5.45244	1.32218
dx	6.4071	1.26161

Chi^2	R^2
0.02367	0.98173

Still Image for Video S1

A single *C. reinhardtii* cell is optically trapped by a 785 nm laser. No loss of activity of the biflagellate *C. reinhardtii* cell observed after an extended 10 minutes exposure to the above-normal laser power in the laser trap proves the laser-trapping Raman spectroscopic system has little damage to microalgae cells.