Isolating single *Euglena gracilis* cells by glass microfluidics for Raman analysis of paramylon biogenesis

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SUPPORTING INFORMATION

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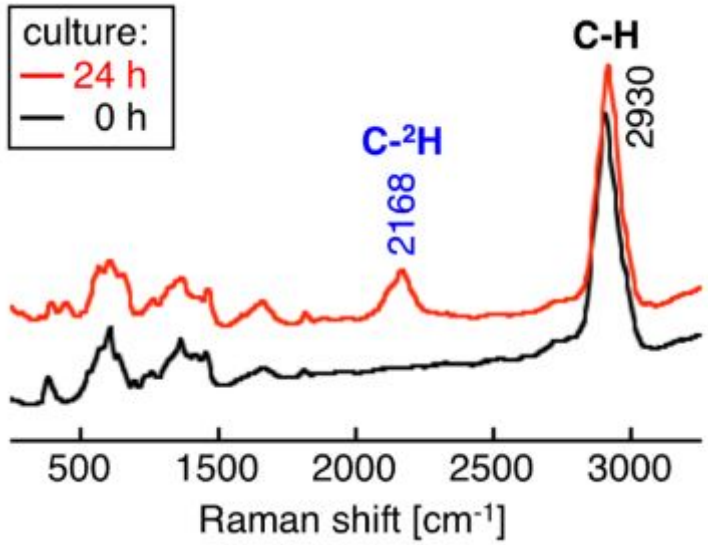

Figure S1. Spontaneous Raman spectra of *E. gracilis* cells incubated with 20% ^2H_2O culture media. The spectra were obtained at (black) 0 h and (red) 24 h of incubation, respectively. The spectra were measured in following conditions after quenching auto-fluorescence. Laser: 532 nm (non-filter). Exposure time: 5 sec. Accumulation: 16.

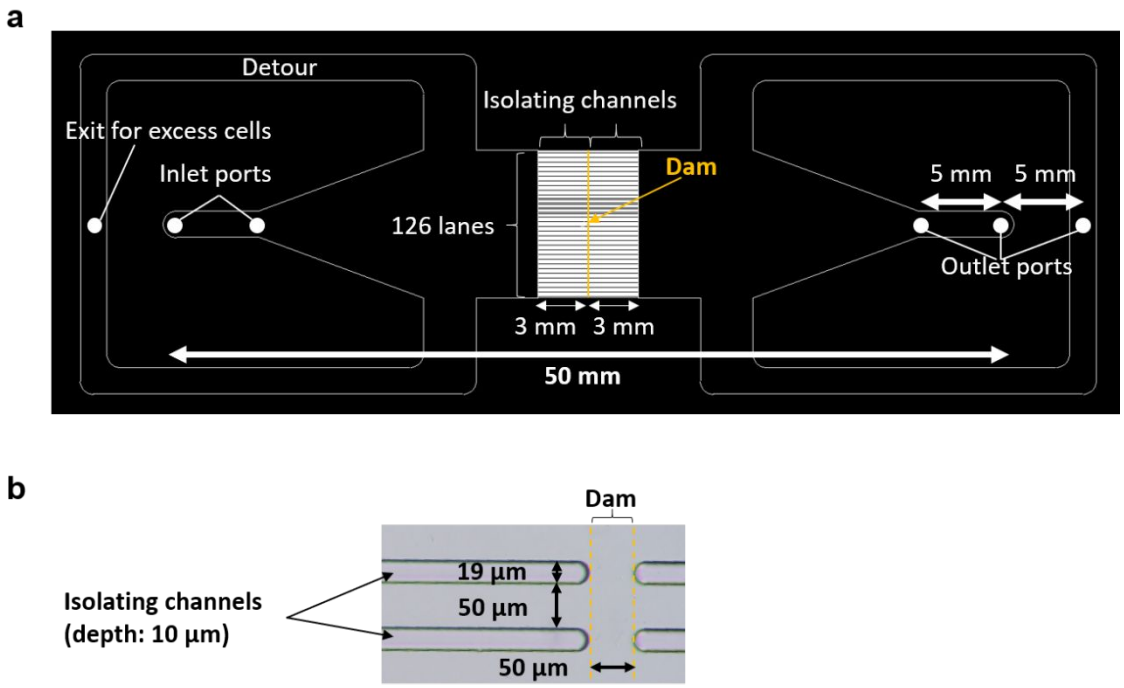

Figure S2. Dimensions of the microfluidic device for *E. gracilis* isolation. (a) Overall design of the device. All ports are 0.8 mm diameter. (b) Magnified image of the dam structure.

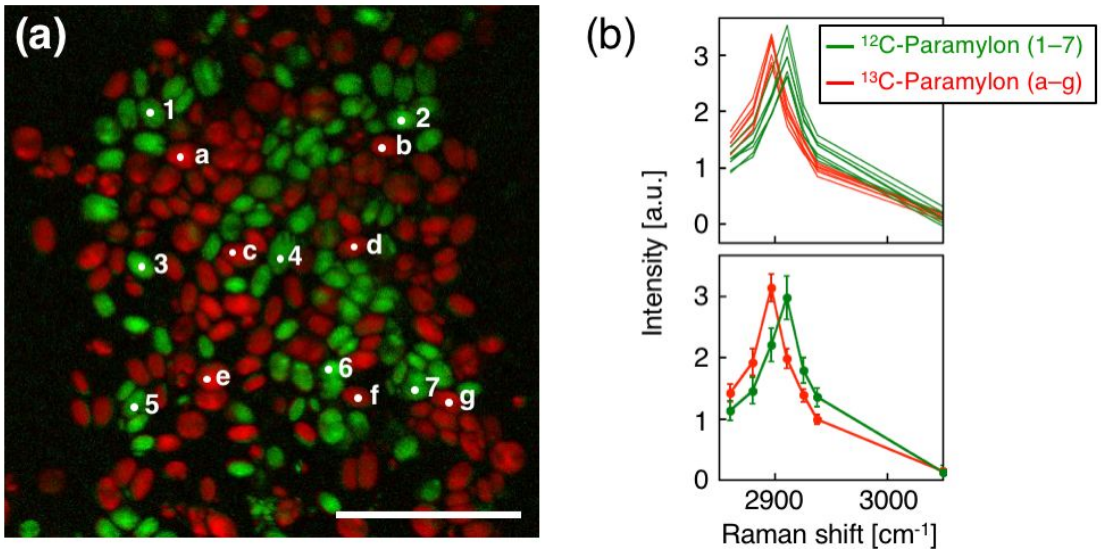

Figure S3. SRS microscopy of extracted ^{13}C- and ^{12}C-paramylons. (a) An SRS image of extracted paramylons (mixture of ^{13}C- and ^{12}C-paramylons). The image is the same as Figure 5c. Pseudo-colors of SRS images are assigned as follows: red, ^{13}C-paramylon; green, ^{12}C-paramylon. Scale bars: 10 μm. (b) Spectral shifts of ^{13}C- and ^{12}C-paramylon. (Top) Confirmation of the spectral shift of ^{13}C-paramylon by repeating the SRS measurement of authentic ^{13}C- and ^{12}C-paramylon samples. The measurement points are shown in Figure S3a (n = 7 for each type of samples). (Bottom) The averaged spectra of ^{13}C- and ^{12}C-paramylon samples plotted at each wavenumber point with the standard deviation.

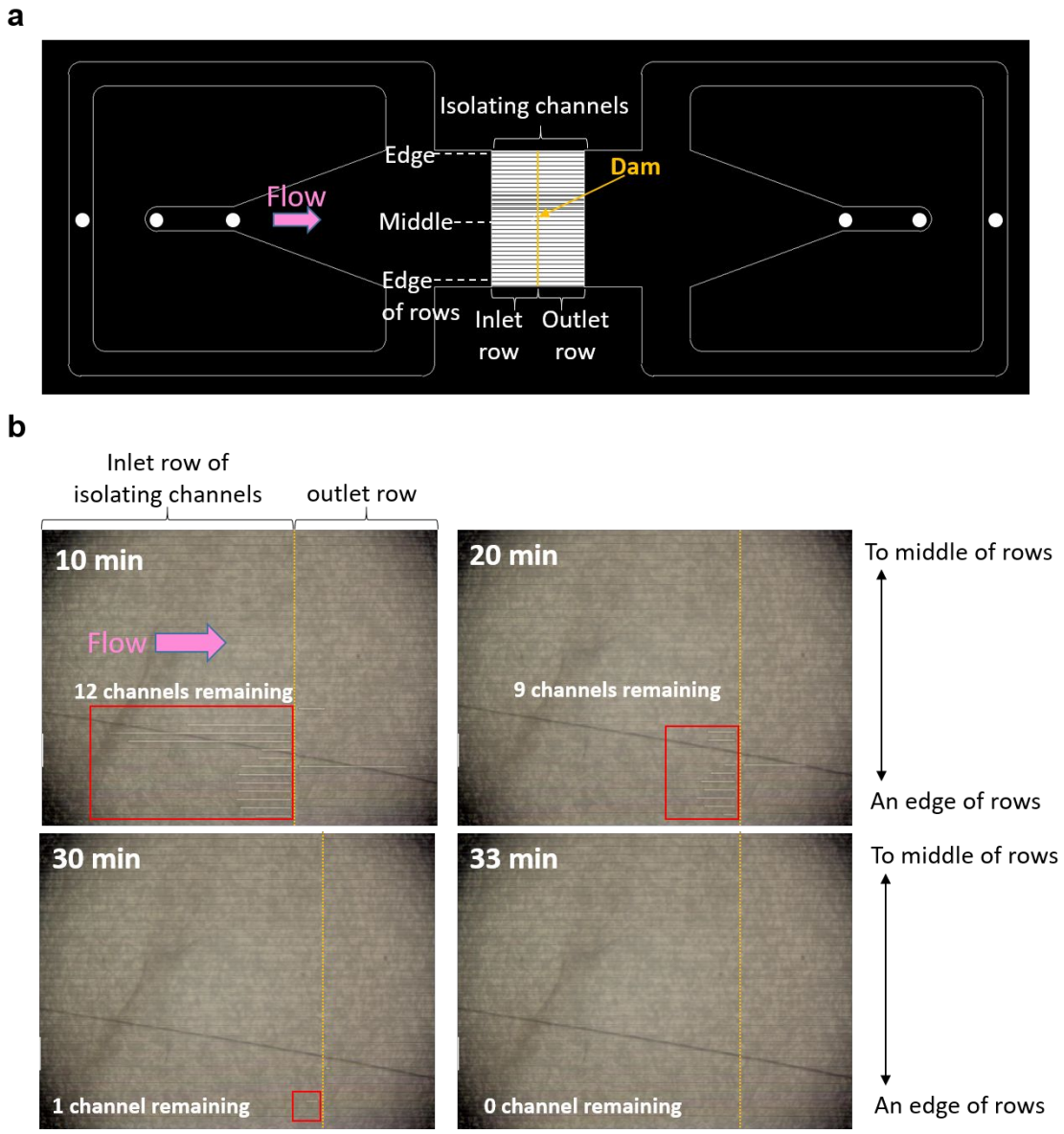

Figure S4. Introduction of water into isolating channels in the semi-closed microfluidic device. (a) Illustration of isolating channels and dam structure on the microfluidic device. (b) Introduction of water into isolating channels and above the dam structure with gap height of 1.1 μm. Water entered the middle of the inlet and outlet rows of the isolating channels first. The number of channels containing air bubbles was reduced as a longer period of water introduction was applied. Flow rate was 50 μL/min. Isolating channels containing air bubbles are enclosed by red rectangles. Dam structure is indicated by the dotted line.

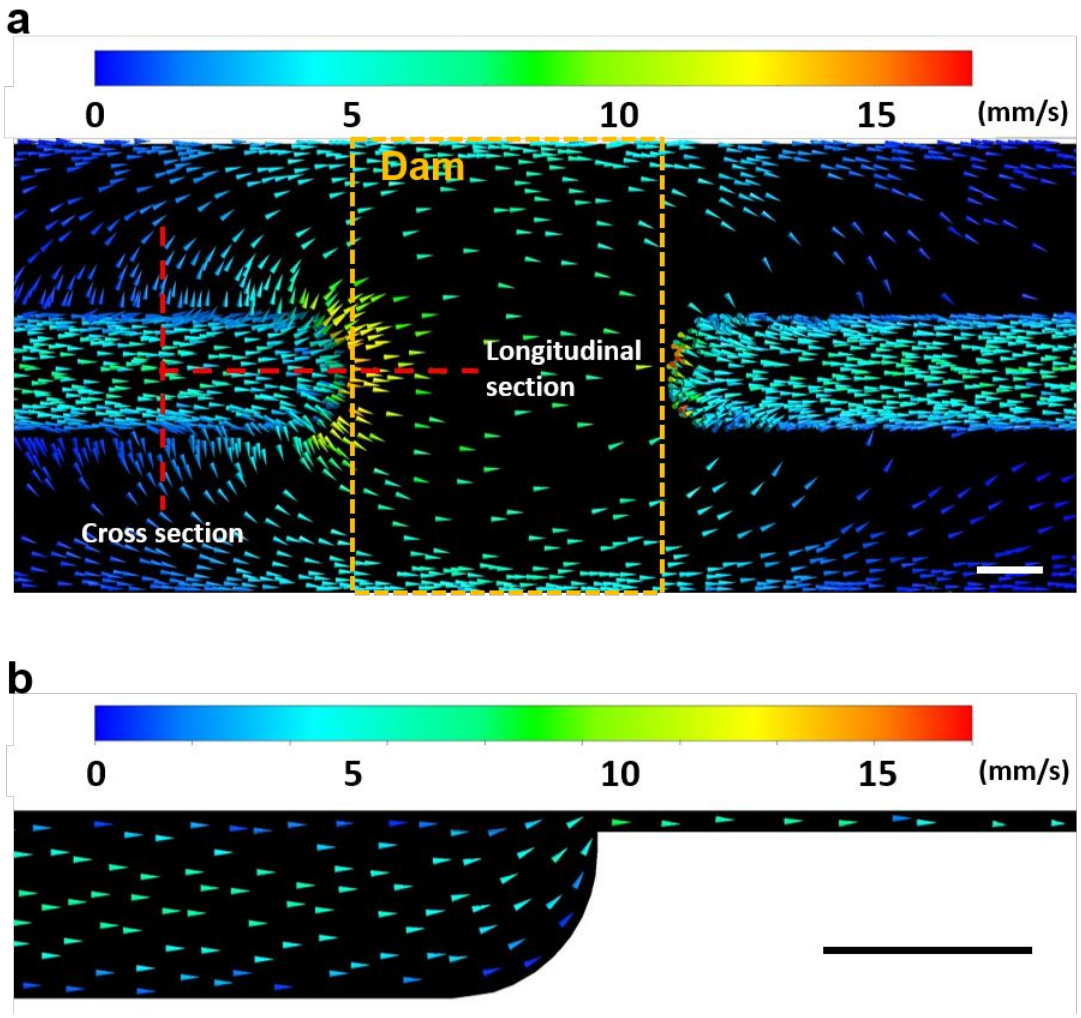

Figure S5. Simulated vectors of flow in an isolating channel. The colours of vectors indicate the velocities in an isolating channel. (a) Vectors of flow in a top view. Dashed lines indicate where other sections of vectors of flow were calculated: a cross section (corresponding to Figure 3c) and a longitudinal section. (b) Vectors of flow in a longitudinal section.

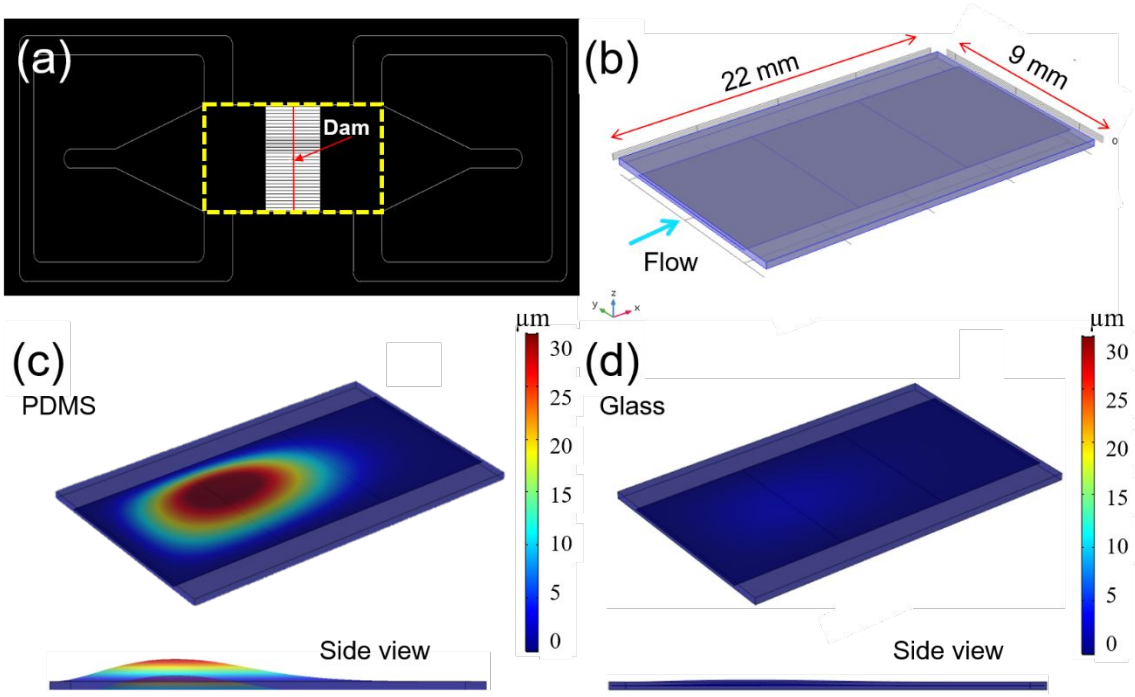

Figure S6. Simulated deformation of glass-based and glass/PDMS-based devices. (A) Simulation domain of the devices indicated by a dashed line. (B) The dimensions of the simulation domain that are 22mm×9 mm×0.42mm (0.2-mm top layer + 0.01-mm channel + 0.2-mm bottom layer) indicated in (A). Top layer (0.2-mm thick) was defined to either a PDMS or a glass layer. Bottom layer (0.2-mm thick) was defined to a fixed layer without deformation. The channel layer (0.01-mm thick) and the dam structure (0.001-mm thick) were used to apply flow. (C) Simulated result of deformation distribution of the PDMS top layer at flow rate of 5 μL/min. (D) Simulated result of deformation distribution of the glass top layer at flow rate of 5 μL/min.

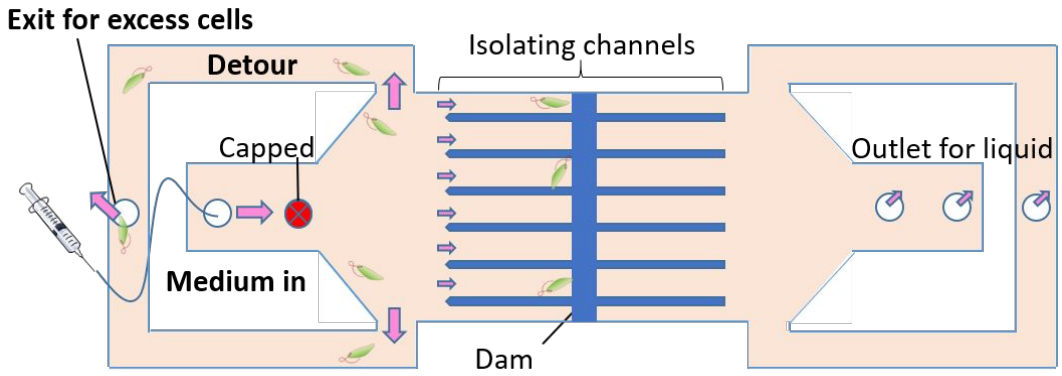

Figure S7. Removal of excess cell in the semi-closed microfluidic device. Flushing by medium removed excess *E. gracilis* cells that were not trapped in isolating channels. Most of the liquid flowed through from the inlet into the detour because of the dam structure.

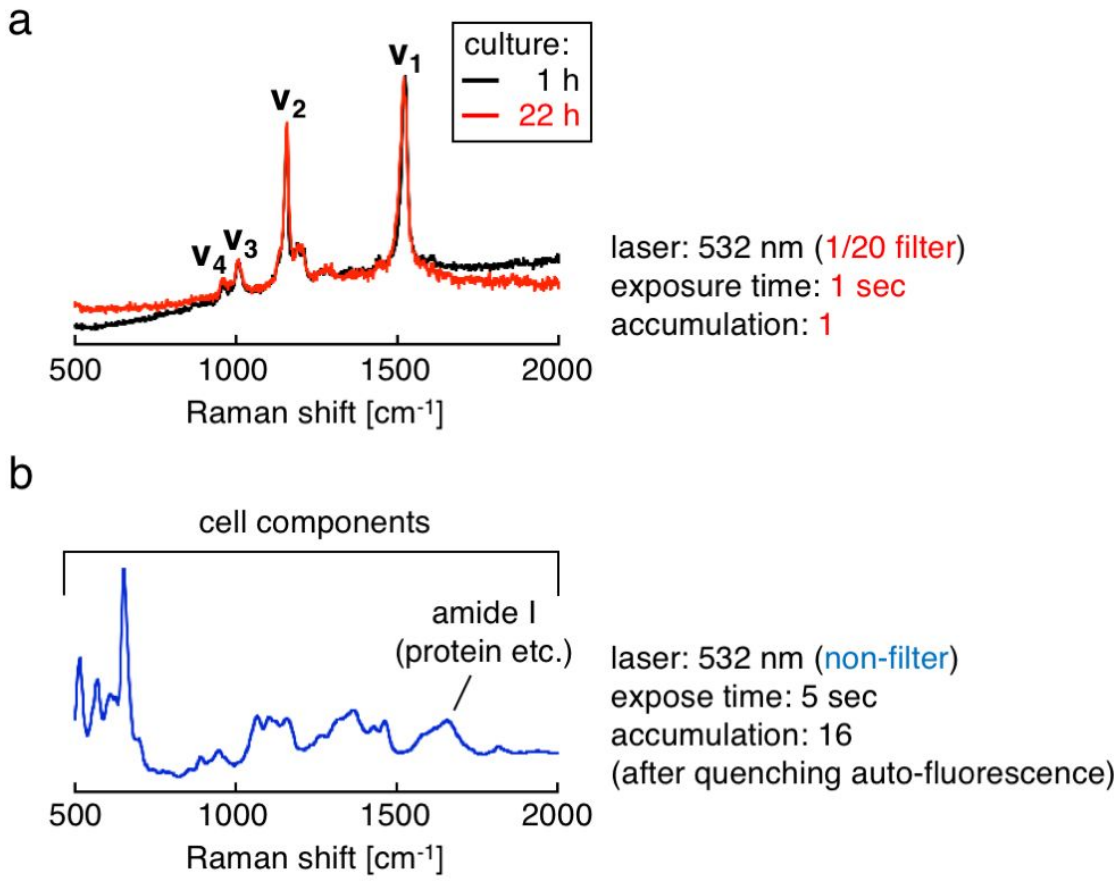

Figure S8. Comparison of Raman spectra of *E. gracilis* cells. (a) Resonance Raman spectra of *E. gracilis* cells incubated with 30% ^2H_2O culture medium in a broad spectral range. These spectra correspond to the spectra in Figure 4a. These spectra show typical resonance Raman signals of carotenoids (ν_1–ν_4 peaks).[34] The conditions of Raman spectroscopy are also shown. (b) Raman spectrum of an *E. gracilis* cell incubated with 30% ^2H_2O culture medium in the same spectral range as (a). The spectrum is superposition of the signals from cell constituents including proteins, nucleotides and lipids. The conditions of Raman spectroscopy are also shown.

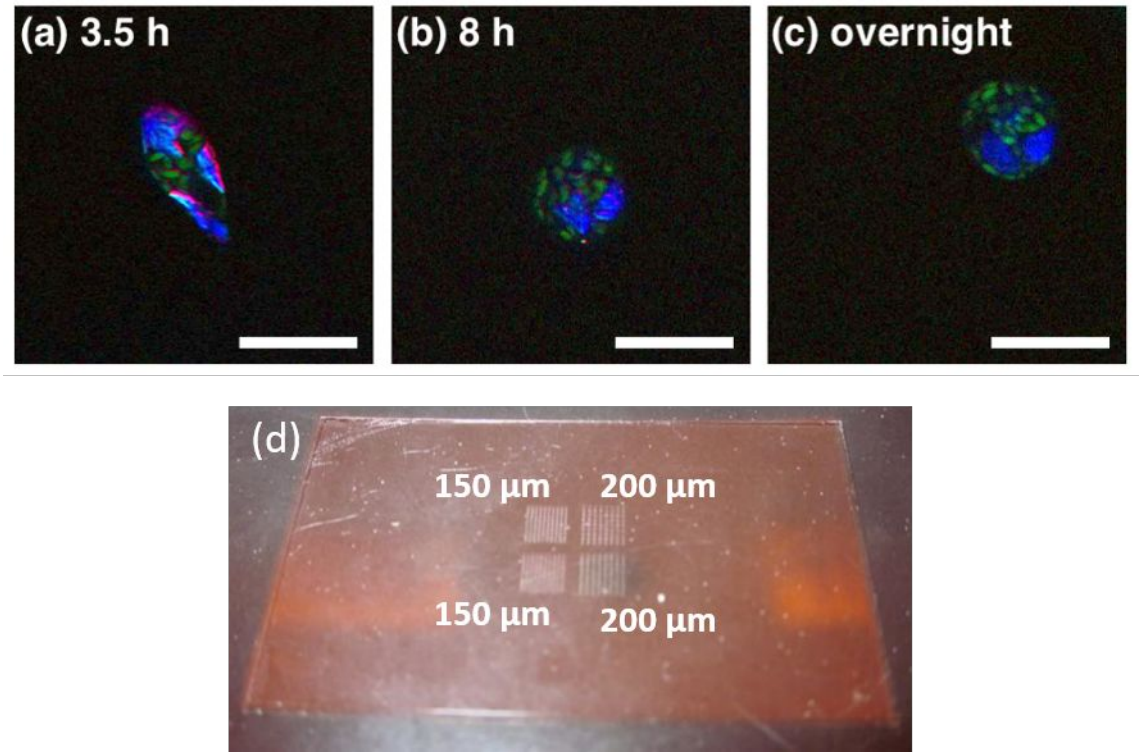

Figure S9. SRS images of an identified single *E. gracilis* cell in an isolating chamber without supply of fresh culture medium. The images were acquired after incubation with paramylon-induction medium containing 20 mM $NaH^{13}CO_3$ for (a) 3.5 h, (b) 8 h and (c) overnight. Pseudo-colors of constituents are assigned as follows: red, ^{13}C-paramylon; green, ^{12}C-paramylon; blue, chlorophyll. Scale bars: 20 µm. (d) A microwell-array device used for this experiment. *E. gracilis* cells were isolated into two sets of 64 wells of 200-µm diameter and two sets of 100 wells of 100-µm diameter. Each well was 30-µm deep.