Measuring neutral lipids in fixed diatom cells using BODIPY 505/515

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We use this protocol and it's working

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ABSTRACT

This protocol is for the measuring neutral lipids in fixed diatom cells using BODIPY 505/515, as developed on the centric diatom *Thalassiosira* spp.

This protocol has been used in the following publications:

Argyle, P. A., Walworth, N. G., Hinners, J., Collins, S., Levine, N. M., & Doblin, M. A. (2021). Multivariate trait analysis reveals diatom plasticity constrained to a reduced set of biological axes. *ISME Communications*, *1*(1), 59.

Argyle, P. A., Hinners, J., Walworth, N. G., Collins, S., Levine, N. M., & Doblin, M. A. (2021). A high-throughput assay for quantifying phenotypic traits of microalgae. *Frontiers in microbiology*, *12*, 706235.

MATERIALS

BODIPY™ 505/515 (4,4-Difluoro-1,3,5,7-Tetramethyl-4-Bora-3a,4a-Diaza-s-Indacene) **Thermo Fisher Catalog #D3921**

Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) **Contributed by users**

Preparation of BODIPY stock solution

1 Add 2 mg of BODIPY™ 505/515 (4,4-Difluoro-1,3,5,7-Tetramethyl-4-Bora-3a,4a-Diaza-s-Indacene) **Thermo Fisher Catalog #D3921** in powder form to 1 mL of DMSO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D1435** to create the stock solution of [M] 2 mg/mL .

Store this in dark glass at -20 °C to prevent degradation of the fluorescent dye.

Diatom sample preparation

10m

2 Sample the diatom culture of interest. For 'tube mode' flow cytometry take minimum 500 µL into an eppendorf tube or other flow cytometry tube. For plate mode take 200 µL into a round-bottom flow cytometry plate.

3 If using live cells, go to step 6

If using fixed cells:

Add [M] 8 % volume Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) **Contributed by users** at [M] 10 % (v/v) to reach a final concentration of approx [M] 0.8 % volume . For example, add 100 µL of Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) **Contributed by users** to 1000 µL of microalgae sample.

4 Agitate to mix, then leave to fix for 00:10:00 to ensure fixation

10m

5 If samples are to be analyzed at a later date, samples should be stored in the fridge (max 48

hours), or flash frozen in Liquid nitrogen **Contributed by users** then stored at -80 °C .

Flow cytometry analysis

6 Measure the background fluorescence of unstained cells using a flow cytometer. This protocol was developed using a CytoFlex LX.

Equipment	
CytoFLEX LX	NAME
Flow cytometer	TYPE
Beckman Coulter	BRAND
C40312	SKU
https://www.beckman.com	LINK
CytoFLEX LX N3-V5-B3-Y5-R3-I2 Flow Cytometer (21 Detectors, 6 Lasers)	SPECIFICATIONS

Fluorescence is measured using 488 nm excitation and 525/40 nm detection.
Measure the background fluorescence of at least 200 cells but ideally 2000 or more.

7 Add the BODIPY stain in a [M] 1 % (v/v) to final concentration of approx [M] 0.002 mg/mL .
For example add 2 µL to 200 µL of microalgae sample, or 2.2 µL to a fixed microalgae sample (200 µL sample with 20 µL
Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) **Contributed by users**
. Remember to take into account volume removed due to flow cytometry analysis.

8 Re-read the sample on the flow cytometer using the same laser parameters. Record the median fluorescence on the x channel for at least 200 cells

9 Calculate the change in median fluorescence between the stained and unstained sample. This is indicative of the level of neutral lipids present in the cell

10 If comparing between taxa of different sizes, size correction may be advisable. This may be done by dividing the change in median fluorescence by the equivalent spherical size, approximated from forward scatter which is measured in tandem during the flow cytometry analysis.