Modified Filter-Transfer-Freeze ("FTF") Technique for Raman Microspectroscopic Analysis of Single Cells Version 2

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Abstract

A method for concentrating aquatic microbes for single-cell analysis by Raman microspectroscopy. Modified from Hewes and Holm-Hansen (1983): A Method for Recovering Nanoplankton from Filters for Identification with the Microscope: The Filter-Transfer-Freeze (FTF) Technique. Limnology and Oceanography. 28(2): 389-394.

Many materials commonly used in microscopy to examine individual microorganisms contribute contaminating Raman scattered emissions, e.g., most types of membranes and filters, glass slides, cover slips, immersion oil, glycerol, etc. and are therefore unsuitable for this application. We developed a protocol that enables single-cell analysis while minimizing extraneous Raman scattered photons. The technique transfers cells from popular polycarbonate membranes to mirror-finished 304 stainless steel slides (1x3x0.0235') supplied by Stainless Supply® (Monroe, NC USA).

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Before start

Additionally, a frozen aluminum block (previously stored at -80°C) is necessary as well as typical lab supplies such as pipettors and forceps.

Note: Polycarbonate membrane filters of various pore sizes can be used.

Materials

Methanol PA-33900HPLCCS4L by P212121

MilliQ water by Contributed by users

Acetone solution 48358 SUPELCO by Sigma Aldrich

Isopropanol by Contributed by users

304 Stainless Steel Sheet SS-304-8-24G by Stainless Supply

Polycarbonate Membrane 0.2µm pore size GTTP02500 by Emd Millipore

Protocol

Prepare Stainless Steel Slides

Step 1.

To begin, completely peel off protective film applied to the polished side of the steel slide by the manufacturer. This is the side where the sample will applied.

Prepare Stainless Steel Slides

Step 2.

Acetone clean in ultrasonic bath for 15 minutes.

Prepare Stainless Steel Slides

Step 3.

Rinse with DI water thoroughly.

Prepare Stainless Steel Slides

Step 4.

Isopropanol clean in ultrasonic bath for 15 minutes.

Prepare Stainless Steel Slides

Step 5.

Rinse with DI water thoroughly.

Prepare Stainless Steel Slides

Step 6.

Methanol clean in ultrasonic bath for 15 minutes.

Prepare Stainless Steel Slides

Step 7.

Rinse with DI water thoroughly.

Prepare Stainless Steel Slides

Step 8.

MilliQ water clean in ultrasonic bath for 15 minutes.

Prepare Stainless Steel Slides

Step 9.

Rinse by MilliQ water thoroughly.

Prepare Stainless Steel Slides

Step 10.

Allow slide to air dry in lint-free environment.

Filter

Step 11.

Concentrate cells from fixed suspension onto white polycarbonate membranes by filtering appropriate volume.

NOTES

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Some fixatives may be problematic for Raman analysis. Formaldehyde (2% final conc.) performs well, but 1-2% glutaraldehyde produces unacceptable fluorescence during Raman interrogation.

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Other fixatives have not been rigorously evaluated.

Rinse

Step 12.

Rinse thoroughly with PBS (if seawater sample) or MilliQ H_2O.

Cut Wedges

Step 13.

Cut filter into wedges of desired size with EtOH-sterilized scissors, indicating with pencil marks the top side of the filter.

Hybridize

Step 14.

If desired, subject filter wedge to standard fluorescent in situ hybridization (oligo-FISH) protocols with oligonucleotide probes of your choosing to selectively observe specific taxa.

NOTES

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Samples prepared by catalyzed reporter deposition FISH (CARD-FISH) have **not** been evaluated for Raman microspectroscopic analysis and may present special challenges.

Prepare for Transfer

Step 15.

Place a droplet of sterile MilliQ H_2O (2-5 µl depending on wedge size) onto polished side of stainless steel slide, near the edge.

Transfer

Step 16.

Place the filter wedge upside down on the droplet (see photo), making sure an edge of the wedge is hanging off the slide.

Freeze

Step 17.

Place the entire stainless steel slide on a frozen aluminum block (-80°C).

Remove filter

Step 18.

Once the droplet is frozen (after a few seconds), quickly peel the filter wedge away from surface with forceps. Cells are now transferred to stainless steel. Allow sample to dry at room temperature in the dark and store frozen until ready for Raman microspectroscopic surveys.

NOTES

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Transfer efficiency of cells from filter wedge will vary depending on a number of variables, e.g., cell types, debris on the filter, mode of preservation, etc. With *Synechococcus* sp. cells, for example, we have routinely achieved transfer efficiencies of 51 - 77% to mirror-finished stainless steel slides. Your results may vary.