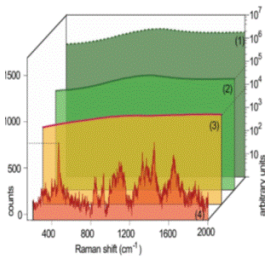
Feb 25, 2020

New chemiphotobleaching protocol for Raman spectroscopy



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Protocol status: Working
We use this protocol in our group for diverse set of samples and it is working.

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Abstract

We present a new and simple chemiphotobleaching method to irreversibly suppress background fluorescence of biological specimens during sample preparation for Raman microspectroscopic analysis. Our method combines mild hydrogen peroxide oxidation with broad spectrum visible light irradiation of the entire specimen. We show that >99% of the entire sample's background fluorescence is eliminated after simultaneous exposure to a low concentration of hydrogen peroxide (3%) and irradiation by a standard photodiode lamp for 0.5–2 hours.This treatment permits observing intracellular distributions of macromolecular pools, isotopic tracers, and even viral propagation within cells previously not amenable to Raman microspectroscopic examination.

Attachments

PDF

Tear Down the Fluore...

1.3MB

Guidelines

The level of background fluorescence of biological materials is sample-dependent. For each new sample type, the chemiphotobleaching time should be empirically optimized. In some highly recalcitrant fluorescent samples or in cases of limited sample availability which may preclude optimizing treatment time, we advocate chemiphotobleaching for 10 h. In rare cases where samples have residual fluorescence, the entire cell is subjected to brief (1–8 min) laser photobleaching on the Raman microspectroscope stage to totally quench fluorescence. Our approach is significantly less harmful to biological specimens than previously published chemical bleaching methods and requires no modification of standard Raman microspectrophotometers.

Materials

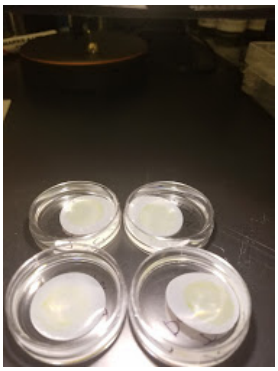
MATERIALS

- Polycarbonate Membrane 0.2µm pore size **Merck Millipore (EMD Millipore) Catalog #**GTTP02500
- 3% Hydrogen Peroxide (H2O2) Solution, Lab Grade **Boster Bio Catalog #**AR1108
- formaldehyde **Fisher Scientific Catalog #**F79

1 Fix cells suspension with 2% final concentration of formaldehyde for 15 min.

2 Concentrate cells from fixed suspension onto GTTP 0.2 um membranes by filtering appropriate volume. (depend on cells density)

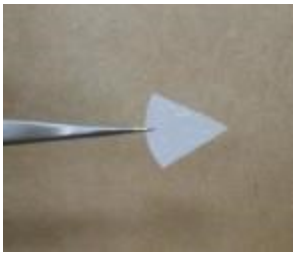
3 Put the filter in a small petri dish and fill in with 2 ml of 3 % Hydrogen Peroxide.



4 Put the petri dish with the filter under bright white (3695) direct light (60 Lumens per Watt) (regular desk LED light lamp) for 0.5 – 3 hours. The distance between the light source and petri dish should be 5-10 cm.

5 Filter-Transfer-Freeze bleached sample to the mirror-finished stainless steel microscope slide (*Taylor et al., 2017;* dx.doi.org/10.17504/protocols.io.g4qbyvw*):*

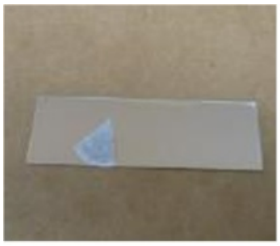
5.1 Prepare filter wedges of GTTP membranes and mirror-finished stainless steel microscope slide:

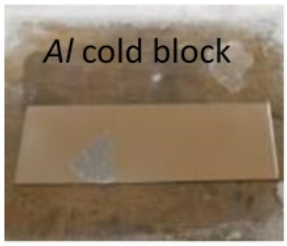


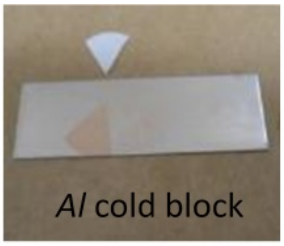
5.2 Place 5 ul sterile MilliQ water on a clean mirror-finished stainless steel microscope slide and then wedge sample side down.

5.3 Place mirror-finished stainless steel microscope slide and filter on -80°C chilled aluminium block. After ~20 sec, peel membrane away and cells remain frozen to slide.

Return slide to RT and air dry.







6 Perform Raman spectroscopy measurements.