FEB 25, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bchqit5w

External link:
<https://www.nature.com/articles/s41598-019-52321-3>

Protocol Citation: Elena Yakubovskaya, Tatiana Zaliznyak, Joaquin Martinez Martinez, Gordon T Taylor 2020. New chemiphotobleaching protocol for Raman spectroscopy. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bchqit5w>

MANUSCRIPT CITATION: Yakubovskaya, E., Zaliznyak, T., Martínez Martínez, J. et al. Tear Down the Fluorescent Curtain: A New Fluorescence Suppression Method for Raman Microspectroscopic Analyses. *Sci Rep* 9, 15785 (2019). <https://doi.org/10.1038/s41598-019-52321-3>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

New chemiphotobleaching protocol for Raman spectroscopy

Elena Yakubovskaya¹, Tatiana Zaliznyak¹,
 Joaquin Martinez Martinez², Gordon T Taylor¹

¹School of Marine & Atmospheric Sciences, Stony Brook University;

²Bigelow Laboratory for Ocean Sciences

TaylorLab

Elena Yakubovskaya

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

[Tear Down the Fluorescent Curtain.pdf](#)

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.

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

Created: Feb 12, 2020

Last Modified: Feb 25, 2020

PROTOCOL integer ID:
33040

Keywords: Raman spectroscopy, laser-induced background fluorescence, bleaching, hydrogen peroxide

MATERIALS

MATERIALS

⊗ Polycarbonate Membrane 0.2µm pore size **Emd Millipore Catalog #GTP02500**

⊗ 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 GTP 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](https://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.