Staining for BromodeoxyUridine (BrdU) Immunohistochemistry

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We routinely use 10% neutral buffered formalin for fixing our BrdU treated tissues (fix for 24 to 48 hours). This procedure is the one we use on paraffin sections. It will also work quite well on cultured cells and frozen sections with a few minor modifications.

A recent reference to our technique is:

Rose, M.L., D.R. Germolec, R. Schoonhoven, and R.G. Thurman (1997). Kupffer cells are casually responsible for the mitogenic effect of peroxisome proliferators. Carcinogenesis 18(8): 1453-1456.

Staining procedure:

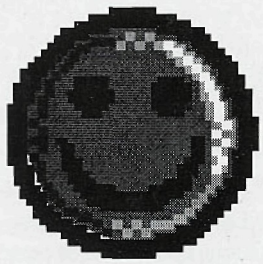
BrdU Immunohistochemistry (using the DAKO Envision kit): the antibody is anti-Bromodeoxyuridine, Bu20a from Dako cat # Mo744.

1) Hydrate slides to double distilled water as per standard procedures.
2) Hydrolyze with 4N HCl at 37°C for 20 minutes. All of the remaining steps are performed at room temperature.
3) Rinse twice with double distilled water, 1 minute each rinse.
4) Rinse twice with phosphate buffered saline (standard rinse and holding buffer) plus 1% Tween 20, 3 minutes each rinse.
5) Blocking reagent. H_2O_2 (blocking step for endogenous peroxidase), 5 minutes.
6) Repeat step 4.
7) Primary antibody (anti-BrdU) incubate for 10 minutes, 1:200 dilution (20 minute incubation time for preputial gland only).
8) Repeat step 4.
9) Polymer labeled secondary antibody[1], incubate for 10 minutes (15 minute incubation time for preputial gland only).
10) Rinse well with double distilled water.
11) Incubate with working DAB solution (1 drop DAB per 1 mL buffer) for 8 minutes.
12) Rinse well with double distilled water.
13) DAB Enhancer[2] solution, incubate for 5 minutes.
14) Rinse well with double distilled water.
15) Stain with Aqua Hematoxylin[3] for 35 seconds and rinse with tap water.
16) Place in tap water for 5 minutes.

Dehydrate and coverslip according to standard procedures.

We always include a portion of small intestine (because of the high rate of cell turnover) from the same animal with all tissues to look at for cell proliferation. The rapid cell turnover makes for a great positive control. Also, if it is from the same animal then it is easy to know if the osmotic pump failed, *i.e.*, no staining.

1. Part of the DAKO Envision Kit (TM; catalog #K1392); contains prediluted blocking reagent, polymer-labeled secondary antibody, and DAB.
2. DAB Enhancer™ is available from Innovex Biosciences (catalog #NB308).
3. Aqua Hematoxylin™ is also from Innovex (catalog #NB305A).



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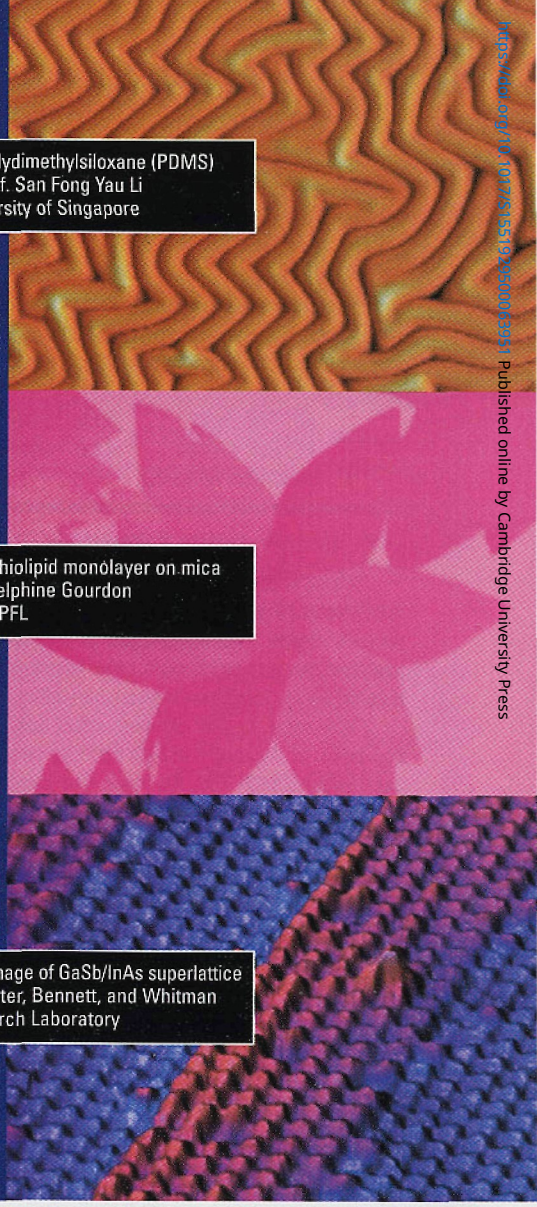

A 6µm AFM image of polydimethylsiloxane (PDMS)
Courtesy of Prof. San Fong Yau Li
National University of Singapore

A 14µm LFM image of a thiolipid monolayer on mica
Courtesy of Delphine Gourdon
EPFL

A 300Å cross section STM image of GaSb/InAs superlattice
Courtesy of Barvosa-Carter, Bennett, and Whitman
Naval Research Laboratory

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