



Tech Report 203:

Light and Electron Microscopy Techniques

For Use with Flex I[®] Culture Plates

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Culturing Cells in a Mechanically Active Environment[™]
Flexcell International Corporation • 437 Dimmocks Mill Road, Suite 28 • Hillsborough, NC 27278
800-728-3714 • (919) 732-1591 • FAX: (919) 732-5196 • www.flexcellint.com

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LIGHT MICROSCOPY HISTOCHEMICAL STAINING

BRIGHT FIELD, PHASE CONTRAST, AND POLARIZED LIGHT

1. Giesma Stain for general staining of cells cultured on Flex I® membranes.
 - a. Fix cells with acetic methanol (1 part glacial acetic acid, 3 parts neat methanol) for 5 minutes at 25 °C.
 - b. Aspirate the fluid and add 1% Giesma stain in acetic methanol (filter through 0.22 micron filter immediately prior to use). Stain for 2 minutes at 25 °C.
 - c. Aspirate stain and save (you can re-use the stain if no radioactive material was used with the cells). Refilter before use.
 - d. Wash cells gently with 60% methanol in water for 1-2 minutes. Aspirate and repeat. Cells will stain bright red.
 - e. Remove rubber well from the plastic plate. Cut into wedges, apply a coverslip and view with a light microscope.

***NOTE:** For general cell staining, this method works well and does not compromise cell attachment to the rubber.*

2. Other stains, such as crystal violet, Von Kossa for calcium phosphate or alizarin red calcium may be used.
 - a. In the latter cases, a non-acidic fixative is best and 60% methanol can be used. Cells sheets may crack but cells can be stored indefinitely in this state.
 - b. Other fixatives recommended are 2% neutral buffered formalin or 4% paraformaldehyde.

EPIFLUORESCENCE

1. The silicone elastomer has little or no background fluorescence at wavelengths used for fluorescein and rhodamine fluorophores.
2. Follow the same procedures as in A above.
3. Be sure to use nonfluorescing mounting media and immersion oil with glass cover slips.
4. The Flex I® membranes may be cut into wedges and cemented to glass slides with super glue or silicone adhesives. A bubble of the fluorescent stain or other dyes can be carefully added to the top surface of the wedge. This technique can be useful when several fluorescent probes or other stains are needed on the same population of cells. It can also reduce costs by limiting the amount of the fluorophore needed.

TRANSMISSION ELECTRON MICROSCOPY SAMPLE PREPARATION

FIXATION

1. Medium should be aspirated from the cells, and the cell sheets washed gently with pre-warmed (37 °C) phosphate buffered saline (PBS).
2. PBS is aspirated and the cell sheet fixed with 4% paraformaldehyde in 0.1 cacodylate buffer pH 7.4 for 5 minutes.
3. Cells may be post-fixed with 2% osmium tetroxide buffered with cacodylate to pH 7.4. Cells are then washed twice with cacodylate buffer.

DEHYDRATION

Cells are dehydrated through a graded series of alcohols (50-100%).



EMBEDDING

1. Cells are embedded with EPON or other embedding medium while still on the rubber membrane in the well of the Flex I® plate.
2. When embedding medium has cured, remove the EPON block from the culture plate well as a disc.
3. Cells are oriented on the bottom side of the EPON disc and may be cut directly from the disc or smaller pieces of the disc made, maintaining orientation from the perimeter to the center and sections then made.

SCANNING ELECTRON MICROSCOPY

FIXATION

Cells may be washed and fixed as above down to and including the graded ethanol series steps.

DEHYDRATION

Further desiccation is carried out by treatment of the cell sheets with hexamethyldisilazane (HMDS) for 5-30 seconds. Specimens can be air dried in a fume hood at room temperature.

SECTIONING

Sections of the rubber membrane may be cut with cells attached, mounted on aluminum studs, sputter coated with gold-palladium and then analyzed by scanning electron microscopy.