

# Pagano Lab – Protocol

## Photosensitization of Fluorescent Lipids for EM Studies

Example using Fluorescent Cer

(see Pagano et al., *J. Cell Biol.* 1989 109:2067-2079 and *J. Cell Biol.* 1991 113:1267-1279.

### **Solutions:**

- 1 M Tris, pH 7.6;
- 1.5 mg DAB/ml 0.1 M Tris, pH 7.6, freshly prepared and kept on ice;
- 0.1 M Na cacodylate, pH 7.4;
- 1% OsO<sub>4</sub> in 0.1 M Na cacodylate, pH 7.4.

### **Steps:**

1. **Cautionary Note:** DAB is a potent carcinogen. Always wear gloves when handling DAB solutions and immerse all materials that contact DAB in bleach before disposing of them;
2. Cells should be grown in 35 mm diameter plastic tissue culture dishes, not on glass cover slips;
3. Label living or fixed cells according to desired protocol (e.g., as above). Living cells should be fixed after labeling (see III B). Always include a sample which has not been treated with the fluorescent lipid as a control;
4. Wash cells in 0.1 M Tris (pH 7.6) and add 0.9 ml DAB solution to the culture dish. Cover dish and place in the dark at room temperature for  $\geq 10$  min;
5. Irradiate sample for 30 min at room temperature using the 476.5 nm line of an Argon laser operating at 50 mW power. To obtain a large area of irradiated cells, the laser beam is expanded to a line  $\sim 1$  mm wide X 1 cm long using a cylindrical lens. [Alternatively, the specimen may be irradiated using a 6.3X objective and filters appropriate for NBD-fluorescence (Zeiss #487717), although only a very small area of the culture dish ( $\leq 1$  mm diameter) is irradiated.]
6. After irradiation, the sample is washed  $\geq 5$  times in 0.1 M Tris (pH 7.6) and observed using phase optics for evidence of a DAB reaction product. Using a dissecting scope and a needle, circumscribe on the inside of the culture dish the region of cells which are DAB-positive;
7. Rinse the sample in 0.1 M cacodylate buffer (pH 7.4) and treat with 1% OsO<sub>4</sub> in 0.1 M cacodylate buffer for 60 min at room temperature;
8. Wash in cacodylate buffer, dehydrate, and embed;
9. After polymerization is complete, the scratch made in Step 6 should readily identify the area of DAB-positive cells. This region of the dish is cut out and mounted for thin section electron microscopy. A typical result using this procedure on human skin fibroblasts can be found in the references noted above.