Labeling cells with DiI:

Preparing DiI solution:

1. Make a stock solution with 50 _g CellTracker CM DiI (Molecular Probes C-7000) and 25 _L DMSO (concentration is 2 mg/mL or about 2 mM).
2. Dilute entire 25 _L of stock solution with 25 mL Hanks' BSS with Phenol Red (do not use Ca^{++} Mg^{++} free variety). Final working concentration is about 2_M.
3. Store unused working DiI solution in 4°C covered with foil.

Labeling cells in vitro with DiI:

1. Discard old medium.
2. Add approximately 6 mL (or between 4 mL and 10 mL) of prepared working DiI solution to flask. Distribute evenly.
3. Incubate at 37°C for 5 min or less, and then at 4°C for additional 15 min.
4. Discard DiI solution.
5. Wash with 5 mL Ca/Mg-free PBS with 0.04% EDTA for no more than 30 sec.
6. Add 4 mL Trypsin for no more than 5 min, make sure it is evenly distributed. Monitor for cell detachment. Slap flask forcefully once if necessary.
7. Add 8 mL (twice the volume of Trypsin) of complete medium.
8. Transfer entire content to a 15 mL conical centrifuge tube.
9. Centrifuge at 1000 rpm for 2.5 min.
10. Discard supernatant.
11. Resuspend pellet in 12 mL complete medium.
12. Aliquot 2 mL per well in a six-well plate with a cover slip in each well.
13. Incubate at 33°C overnight or until adequate cell growth is observed.

Note: Cells treated with DiI can be fixed with paraformaldehyde, permeabilized with cold acetone or 0.2% Triton. Also DiI can be photoconverted using DAB when EM imaging is needed (Histochem Cytochem 38, 725 (1990) and J Neurosci Meth 64, 47 (1996)).